

Characterization of mosquito community assemblages and vector species  
ecology to inform control interventions at hotel settings in Zanzibar

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# Characterization of mosquito community assemblages and vector species ecology to inform control interventions at hotel settings in Zanzibar.

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## RESEARCH OUTPUTS

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## **LIST OF ABBREVIATIONS**

CDC – Centre for Disease Control and Prevention

CHIKV- Chikungunya virus

COE – Carboxylesterases

DALYs – Disability-adjusted life years

DDT - Dichloro-diphenyl-trichloroethane

DENV – Dengue virus

EIN- Ecological interaction networks

EIP - Extrinsic incubation period

ENS – Effective number of species

GDP – Gross Domestic Product

GST – Glutathione-S-transferase

IRS – Indoor Residual Spraying

JEV – Japanese encephalitis virus

kdr – knockdown resistance

LLINs – Long Lasting Insecticide Treated Nets

MBD(s) – Mosquito-Borne Disease(s)

MODIS - Moderate-resolution imaging spectroradiometer sensors

MSW – Municipal solid waste

NcEC – Non-chemical Environmental Control

NDVI - Normalized difference vegetation index

PCR – Polymerase Chain Reaction

RIDL – Release of insect with dominant lethal trait

RVFV- Rift valley fever virus

SIT – Sterile insect technique

SNP -Single nucleotide polymorphism

VBDs – Vector-borne diseases

VGSC – voltage-gated sodium channel

WNV – West Nile virus

YFV – Yellow fever virus

ZAHREC – Zanzibar Health Research Ethics Review Committee

ZAMEP – Zanzibar Malaria Elimination Programme

ZATI – Zanzibar Association of Tourism Investors

ZIKV – Zika virus

## GLOSSARY OF SELECTED TERMS

**Anautogeny** – Refers to females of blood-sucking insects that require at least one blood-meal to develop their eggs.

**Anthropophagy** – Female or male hematophagous arthropod that feed on humans.

**Anthropophily** – Female or male hematophagous arthropod that prefer to feed on human compared to other animals.

**Autogeny** – Refers to females of blood-sucking insects that lay at least the first batch of eggs without a blood-meal. However, a blood-meal is required for each subsequent oviposition.

**Endophagic** – Insect that enters houses to blood-feed.

**Endophilic** – Insect that rests inside houses during blood digestion and eggs development.

**Exophagic** – Insect that feeds outside houses.

**Exophilic** – Insect that rests outside houses during blood digestion and eggs development.

**Gonotrophic cycle** – Time from first blood meal to oviposition, and subsequently between successive ovipositions. It is also called the ovarian cycle.

**Mammalophilic** – Insect that prefers to feed frequently on mammals compared with other animals.

**Ornithophilic** – Insect that feeds frequently on birds compared to other animals.

**Zoonosis** – A disease that can be transmitted naturally from a non-human to humans.

**Zoophagic** – Insect that feeds on animals.

## **DECLARATION**

I declare that the thesis, which I hereby submit for the degree of the Doctor of Philosophy in Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

## **ETHICS STATEMENT**

The author, whose name appears on the title page of this thesis, has obtained, for the research described in this work, the applicable research ethics approval.

The author declares that he has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

Ayubo Amisse Kampango



Pretoria, March 2023

## DEDICATION

### **To my family**

My wife Ana Paula, for her love, friendship, endurance, and daily support during this project

To my parents, brother and sister for the encouragements, motivation, and the time they lent  
me to do this project

To my late brother Filipe who is forever in my memory and heart

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## THESIS SUMMARY

Zanzibar archipelago has made significant advances in reducing the burden of mosquito-borne diseases (MBDs). However, the archipelago is still affected by residual foci of malaria outbreaks two decades after being declared a near elimination region. Widespread physiological and behavioural resistance in the vector populations is one of the main causes of postponed malaria elimination in the region. In addition, an increasing body of hospital surveys suggest that life-threatening mosquito-borne viral diseases, notably dengue and chikungunya, are being transmitted silently among foreigners and Zanzibar residents. These findings corroborate previous studies pointing to a higher risk of re-emergence of mosquito-borne viral outbreaks in Zanzibar. Rapid demographic growth coupled with high connectivity between Zanzibar and other endemic regions may exacerbate the risk of explosive epidemics with detrimental impact on the Zanzibar economy and health system. The tourism industry is among the main driving factors of economic and demographic growth of Zanzibar. However, evidence has shown that tourism activities also create the landscape for emergence of MBD outbreaks either by the introduction of infected hosts and vectors, or by causing environmental changes, notably accumulation of solid waste that is conducive to proliferation of mosquito vectors and, eventually, pathogen transmission. The human-mediated introduction of pathogens via tourism has the potential to trigger epidemics of novel MBDs with devastating economic consequences, as observed in other tourism-dependent economies. Therefore, understanding the extent to which tourism associated activities represent a risk of MBD epidemics in Zanzibar is crucial for the design and implementation of measures that reduce the tension between tourism promoted economic growth and risk of MBD epidemics. As such, the works comprising this thesis results from a long quest to identify and characterize tourism associated foci of potential MBD epidemic risks in Zanzibar and use the produced knowledge to design a framework for hotel-based risk assessment and evaluation of innovative approaches for larval sources reduction.

This thesis comprises six chapters. In introductory Chapter 1 the global situation of MBDs, and determinants of transmission and expansion are reviewed. Relevant ecological aspects of three major mosquito vector taxa from the standpoint of pathogen transmission are also presented. In addition, traditional and innovative vector control measures are summarized. This chapter ends with updated information about MBDs in Zanzibar and discusses potential links between tourism activities and MBD risk in Zanzibar. In Chapter 2 the structure and environmental

correlates of mosquito community assemblages at hotels and the degree of vulnerability to mosquito larval habitat removal is reported. The findings demonstrate that hotels in Zanzibar provide a suitable environment for production and maintenance of highly important mosquito vector species. Analysis of mosquito community vulnerabilities to systematic loss of larval habitats indicates that community assemblages can be rapidly disrupted by removal of larval habitats sustaining high species diversity. In Chapter 3, investigations were focused on *Aedes* mosquito taxa to characterize local environment associated drivers of *Aedes* mosquito occurrence and abundance, with emphasis on the vector species *Ae. aegypti* and *Ae. bormeliae*. The findings show that the main sources of these two vectors are improperly disposed small and medium sized plastic containers, and containers used in gardening tasks. Furthermore, open spaces, gardens and residential areas for hotel staff are associated with higher abundance of vector species. The status of insecticide susceptibility in *Ae. aegypti*, the most dominant vector species identified at the hotels is reported in Chapter 4. An overview of mosquito control practices at the hotels indicate that the key mosquito control practices have been residual spraying and open spaces fumigation with insecticides of the classes pyrethroids, carbamates and organophosphates. The findings indicate that the phenotypic resistance to DDT (all hotels) and deltamethrin (some hotels) observed in *Ae. aegypti* does not align with the limited presence of *kdr* mutations, as gene allele V1016G was found in only five of 541 specimens analysed. These findings open avenues for further investigations into causes of DDT/deltamethrin resistance and consequences of an emerging V1016G mutation in the *Ae. aegypti* population of Zanzibar. In Chapter 5 a framework for evaluating the impact of larval source reduction when used as an intervention against mosquitoes at hotel compounds is proposed. A summarising discussion and conclusion are presented in Chapter 6. This thesis increases our knowledge on the ecology of mosquito vectors in Zanzibar and reveals how the archipelago may act as a potential epicentre of future mosquito-borne viral diseases epidemics in the region. It also provides a basis for designing comprehensive mosquito vector surveillance and emerging epidemic risk assessment in Zanzibar.

# CHAPTER 1

## General introduction

## **Abstract**

This chapter reviews the global situation of mosquito-borne diseases (MBDs) and the interplay between climate and ecosystem on the occurrence and expansion of MBDs in the context of global change.

In the first section, the burden, and global trends of MBDs are reviewed. The second section reviews the role of climate, environmental and socio-demographic changes on the emergence, transmission, and expansion of mosquito-transmitted pathogens. The third section reviews ecological and biogeographical aspects of dominant mosquito vector species. Current knowledge of MBDs in Zanzibar and potential driving factors are described in the fourth section, whereas the fifth section reviews the tourism and emerging MBDs epidemics risk association. The sixth section describes available tools for management and prevention of MBDs, including innovative approaches involving genetically modified organisms and insects. Research questions, overarching goals are presented in the remaining sections of this chapter.

### **1.1. Global situation of mosquito-borne diseases: tiny bites, mass destruction**

Mosquito-borne diseases (MBDs) continue to pose a significant global health burden particularly in socioeconomically disadvantaged countries and communities (WHO, 2017a). More than 80% of the world population is at risk, especially in the tropical and subtropical regions where MBDs, most notably malaria, dengue, yellow fever, chikungunya, Zika, Rift Valley fever and Japanese encephalitis contribute to more than 50% of the burden of vector-borne diseases (VBDs) (WHO, 2017a). There has been an unprecedented increase in the incidence of MBDs worldwide, with some diseases expanding into regions where they had subsided or there were no previous records of occurrence (Colón-González et al., 2021; Kraemer et al., 2019; Ryan et al., 2019; WHO, 2021). A rebound in malaria incidence and mortality rates is observed globally, after a decade of sustained decline (2000-2015) and stagnation (2016 - 2018) (WHO, 2020b). At least 241 million infections and 627,000 deaths occurred in 2020, 94% of which were in Africa (WHO, 2021). This represented an increase of nearly 35% in the global malaria mortality rate compared to 405,000 deaths in 2019. Moreover, mosquito-borne viral disease, most tellingly dengue, yellow fever, zika and chikungunya have emerged as regular threats.

Dengue is the fastest expanding arboviral disease now present in virtually all continents (Brady & Hay, 2020; Puntasecca et al., 2021; Ryan et al., 2019). Dengue incidence increased 30-fold over the last 50-years (since 1970) with an estimated 100 - 400 million new dengue virus (DENV) infections occurring each year (Brady & Hay, 2020; Messina et al., 2019). The disease is now present in 129 countries and remains the most common mosquito-borne viral infection in tropical and subtropical regions, placing more than 3.8 billion people at risk of infection (Pang et al., 2017; Wilder-Smith et al., 2017). Similarly, Zika virus (ZIKV) and chikungunya virus (CHIKV) are also expanding rapidly. In 2016, ZIKV moved from anonymity to a public health emergency of international concern (PHEIC) (Gulland, 2016), as unparalleled numbers of infections, coinciding with cases of severe neonatal neurological deformities, flared up across several regions in the South Pacific (2013) and the Americas (2015) (Cugola et al., 2016; Gulland, 2016; Mayer et al., 2017). Currently, ZIKV transmission has been confirmed in 87 countries (WHO, 2019b). Equally, cases of CHIKV infections have re-emerged since the 2000s and are spreading across Asia and the Americas (Halstead, 2015; Mayer et al., 2017). Over the last decades, CHIKV and ZIKV caused an average annual loss of over 106,000 and 44,000 disability-adjusted life years (DALYs), respectively (2010 and 2019) (Puntasecca et al., 2021).

Recently, epidemics of yellow fever virus (YFV) were reported in Africa (Angola and Democratic Republic of Congo) (Grobbelaar et al., 2016; Ingelbeen et al., 2018) and South America (Cunha et al., 2019), after decades of extraordinary reduction in the number of cases and frequency of outbreaks (Lucey & Gostin, 2016). An estimate of 109,000 severe YFV infections and 51,000 deaths occurred in 2018 alone (Gaythorpe et al., 2021). Japanese encephalitis virus (JEV) is the most important cause of viral encephalitis in Asia (Campbell et al., 2011), with recent expansion to the Far East, the Western Pacific Islands and Northern Australia (WHO, 2018). Nearly 68,000 clinical cases of JEV infections are reported yearly in the world, with fatality rate ranging between 20% and 30%. Infections usually leave 30% to 50% of survivors with neurological or psychiatric sequelae (Campbell et al., 2011; WHO, 2018).

The resurgence and rapid expansion of MBDs underscore the great need for comprehensive, dynamic, rapidly scalable, and effective MBD control practices adjustable to global changes. It also emphasizes the need for development of innovative, integrated and cost-saving strategies for vector control that requires an in-depth understanding of the MBDs transmission systems and ecology of MBDs.

## **1.2. Determinants of mosquito-borne diseases**

### **1.2.1. The climate**

The emergence and distribution of MBDs is strongly modulated by the interplay between climatic, environmental, and socio-demographic factors. However, the combined effect of these factors is poorly understood (Franklinos et al., 2019; Norris, 2004; Reiter, 2008; Sutherst, 2004). Climate and weather shape the suitable distribution for MBD transmission by directly or indirectly affecting biological traits of vectors and pathogens, and therefore, the timing, place, and intensity of transmission (Mordecai et al., 2019; Rocklov & Dubrow, 2020). Mosquito abundance, survival and host-seeking frequency increase with rising temperature up to certain species-specific thermal optima, beyond which vector development and survival decline (Mordecai et al., 2019). Temperature fluctuations also affect the pathogen extrinsic incubation period (EIP), that is, the time required for the pathogen to become infective within the vector. The EIP is dramatically shortened in warmer environments (Mordecai et al., 2019; Stopard et al., 2021). Conversely, the transmission range is greatly limited by lower environmental temperatures. In general, warmer temperatures promote faster development of

vectors and pathogens, leading to more generations per year, and vectors feed more frequently, which increases transmission risk and frequency of epidemics (Reiter, 2008; Rocklov & Dubrow, 2020; Sutherst, 2004). There has been concern that global warming could potentially lead to changes in the distribution of MBDs toward northern and high altitudes regions, which could translate into emergence of new transmission foci (Colón-González et al., 2021; Liu-Helmersson et al., 2016; Ryan et al., 2020; Sutherst, 2004). It could also lengthen transmission season in endemic countries (Colón-González et al., 2021; Rocklov & Dubrow, 2020; Ryan et al., 2020). However, regarding malaria specifically, recent studies indicate that higher temperatures are detrimental to malaria transmission suitability, with a thermal optimum of 25°C (Mordecai et al., 2013; Murdock et al., 2016; Paaijmans et al., 2012). In contrast, the transmission suitability of DENV, ZIKV and several other mosquito-borne viruses is predicted to increase because they have thermal optima of around 29 °C (Mordecai et al., 2020; Ryan et al., 2019).

Precipitation is also an important driver of MBDs. However, its effect on MBD transmission dynamics is complex. Depending on species, mosquitoes may benefit both from presence and absence of rainfall (Campbell-Lendrum et al., 2015; Shocket et al., 2021). On one hand, large amounts of rainfall increase transmission by creating conditions for the existence of new suitable aquatic habitats that sustain mosquito larval stages, and may also expand existing ones, as observed with *Anopheles* mosquito species, such as *An. gambiae s.l.*, which utilise temporary water bodies for reproduction (De Meillon, 1951). This also includes a surge in the population of artificial container developing mosquito species because of high availability of water filled artificial containers (Lowe et al., 2018; Shocket et al., 2021). In contrast, unusually heavy rainfalls overflow and flush larval habitats, causing an immediate decrease in density of several mosquito species (Koenraadt & Harrington, 2008; Paaijmans et al., 2007) and lower risk of disease outbreaks in the short-term, as observed with *Aedes aegypti* (Benedum et al., 2018). However, the density of some vector species, such as *An. funestus s.l.*, can rebound earlier levels only a few days after a rainstorm has stopped or floods have receded (Charlwood & Braganca, 2012). On the other hand, severe rainfall shortage can reduce the density of vectors adapted for colonizing temporary natural larval habitats, such as *Aedes aegypti* (Benedum et al., 2018).

The influence of humidity is also poorly understood. However, evidence indicates that high humidity increases mosquito survival and longevity, with a correlated increase in the probability of pathogen transmission (Lehane, 2005). High humidity also reduces the EIP and

blood-feeding intervals, which in turn affects the intensity of pathogen transmission (Foster & Walker, 2019).

### **1.2.2. The environmental and human-related factors**

The risk of MBD transmission is strongly mediated by environmental changes, such as deforestation for agriculture and logging activities, expansion of urbanized areas, construction of dams and irrigation schemes (Hahn et al., 2014; Norris, 2004; Vittor et al., 2009). These anthropogenic actions are known to exacerbate mosquito-associated problems by creating suitable conditions for emergence/re-emergence of outbreaks, and facilitate geographical expansion of vectors and pathogens (Norris, 2004). For instance, deforestation can cause significant ecological and landscape alterations creating novel and suitable biotypes for vectors and animal wildlife close to human settlements (Hahn et al., 2014; Vittor et al., 2009), often increasing the potential for zoonotic spill over of mosquito-transmitted pathogens (Vinson et al., 2022). Deforestation has been pointed out as one of the main drivers of MBD expansion toward high altitude regions (Kweka et al., 2016; MacDonald & Mordecai, 2019).

Socio-demographic factors such as increasing international travel and commerce have also been key pathways for transportation and propagation of vectors and pathogens into new regions (Eritja et al., 2017). For instance, in 2005, a devastating CHIKV outbreak in India was caused by the introduction of a new CHIKV variant from East Africa (Parola et al., 2006; Yergolkar et al., 2006). Similarly, a reappearance of CHIKV epidemics was reported in 2013 in the Americas, approximately 200 years after the first record in the continent (Halstead, 2015).

## **1.3. Mosquito ecology**

### **1.3.1. Occurrence and distribution**

Mosquitoes have a worldwide distribution, occurring on every continent except in Antarctica (Service, 1993; Wilkerson et al., 2021). A total of 3,591 species divided into two subfamilies, namely Anophelinae and Culicinae, are formally recognized (Harbach, 2021; Wilkerson et al., 2021). The spatial distribution of mosquito species ranges from tropical and subtropical regions, through to the Arctic circle. In these regions they can be found in virtually all ecosystems, including deserts, tropical forests, mountainous regions, plains, salt marshes, tidal zones, tundra and boreal forests (Service, 1993). There have been records of mosquitoes at elevations of more than 3000 metres and depths of 1250 metres below sea level (Eldridge,

2005; Nataša et al., 2021). Therefore, understanding the ecological aspects shaping the occurrence and distribution of different mosquito species is fundamental for devising ecosystem-based control measures (Ferguson et al., 2010; LaDeau et al., 2015).

### 1.3.2. Larval ecology

The life history of mosquitoes is inextricably associated with water. Oviposition and development of all immature stages (egg, larva and pupa) as well as emergence of adult mosquitoes occur in water (Becker et al., 2020). The type of resources exploited for oviposition vary considerably between and within mosquito taxa (Hopkins, 1952; Laird, 1988; Rejmánková et al., 2013). Such heterogeneities, in connection with interactions between abiotic and biotic factors, shape adult mosquito population dynamics and can sometimes be a practical predictor of abundance, diversity, distribution and fitness. Consequently, larval habitat ecology shapes the structure of mosquito communities (Juliano, 2008; Rejmánková et al., 2013).

Mosquitoes can occupy virtually any thinkable type of steadily or slow-moving water (Hopkins, 1952; Laird, 1988). As thoroughly described by Service (Service, 1993), Laird (1988) and Rejmánková (2013), mosquito larval habitats comprise large and usually permanent collections of water, such as swamps, marshes, rice fields and borrow pits, to smaller collections of temporary water such as small pools, puddles, water-filled car tracks, ditches, drains and gullies, water filled plastic bottle caps, and mollusc shells. A variety of natural container habitats such as water-filled tree holes, rock pools, hoof prints, bamboo stumps, bromeliad leaves, pitcher plants, leaf axils, split coconut husks and coconut flower spathes, snail shells, and fallen leaves can lend themselves as larval habitats for several mosquito taxa such as *Anopheles*, *Culex*, *Culiseta*, *Aedes*, *Eretmapodites*, and *Orthopodomyia* (Hopkins, 1952; Laird, 1988; Service, 1993). Man-made container habitats, such as clay pots, water-storage jars, tin cans, discarded utensils, plastic bottle caps, and vehicle tyres may also support larvae of *Aedes*, *Culex*, *Culiseta*, *Eretmapodites*, *Orthopodomyia* and some *Anopheles* (Ishtiaq et al., 2021a). Factors driving oviposition sites selection by gravid females are poorly known. However, the large diversity of mosquito larval habits suggest that these factors may be species-specific and context dependent (Juliano, 2008; Rejmánková et al., 2005). Further evidence also suggests that volatile compounds (kairomones) released from dissolved organic matter, plants, eggs, larvae and pupae from conspecifics and predators play important roles in selection of larval sites (Afify & Galizia, 2015; Asmare et al., 2017). For instance, some *Anopheles* (e.g., *An. funestus* group), *Mansonia*, *Coquillettidia* and *Aedes* species (e.g., *Ae.*

*aegypti*) prefer shaded water bodies, others sunlight-exposed aquatic habitats (e.g., *An. gambiae* complex), whereas some breed successfully in highly polluted water contaminated with excreta or rotting organic material (e.g., *Anopheles arabiensis* and *Culex quinquefasciatus*) (Awolola et al., 2007; Azrag & Mohammed, 2018; Subra, 1981). Moreover, a handful of species breed almost exclusively in brackish or salt water in mangroves (e.g., *An. merus*/*An. melas*) and *An. bwambae* breeds in geothermal springs (Muirhead-Thomson, 2009; White, 1985). Conversely, a fraction of generalist species, mostly in the genera *Aedes*, *Anopheles* and *Culex*, exploit a wider range of habitats of different sizes and nature that are subjected to a range of environmental conditions (Christophers, 1960; Hawley, 1988). The ability to develop in a wider range of suitable water bodies or water-filled containers makes the control of generalist species challenging. However, despite high phenotypic plasticity in adapting to virtually any aquatic environment, mosquito larvae are, for yet poorly known reasons, usually absent from larger extensions of uninterrupted deep water, such as lakes, large rivers and fast flowing waters (Rejmánková et al., 2013; Service, 1993). Therefore, knowing the ecology of mosquito larval stage habitats, and patterns of co-occurrence and segregation of mosquito larvae, may help the design of effective, ecologically sound larval source reduction measures. Some of the more important mosquito larval habitats found in the Afrotropical regions are summarized below (Table 1.1).

**Table 1.1.** Classification of mosquito larval habitats. Adapted from Service (1993) and Wilkerson et al., (2021). Only mosquito genera occurring in the Afrotropical region are indicated.

| Category of habitat      | Type of habitat                                   | Genera                                                                                                                           |
|--------------------------|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| <b>Ground habitat</b>    | Marshes                                           | <i>Aedes, Anopheles, Aedeomyia, Coquillettidia, Culex, Culiseta, Hodgesia, Ficalbia, Mansonia, Mimomyia, Uranotaenia, Lutzia</i> |
|                          | Swamps                                            |                                                                                                                                  |
|                          | Exposed ponds, borrow pits                        |                                                                                                                                  |
|                          | Forest ponds                                      |                                                                                                                                  |
|                          | Ditches                                           |                                                                                                                                  |
|                          | Rice fields                                       | <i>Aedes, Anopheles, Culex</i>                                                                                                   |
|                          | Cesspits, sewage                                  | <i>Culex, Culiseta</i>                                                                                                           |
|                          | Wells                                             | <i>Aedes, Anopheles, Culex</i>                                                                                                   |
|                          | Salt-water marshes                                | <i>Aedes, Anopheles, Culex</i>                                                                                                   |
|                          | Salt-water ponds                                  | <i>Aedes, Anopheles, Culex</i>                                                                                                   |
|                          | Subterranean                                      | <i>Anopheles, Aedes, Culex</i>                                                                                                   |
|                          | Exposed pools and puddles                         | <i>Aedes, Anopheles</i>                                                                                                          |
|                          | Forest pools                                      | <i>Aedes, Anopheles, Culex</i>                                                                                                   |
|                          | Hoof-prints                                       | <i>Aedes, Anopheles</i>                                                                                                          |
|                          | Salt-water pools                                  | <i>Aedes, Culex, Anopheles</i>                                                                                                   |
|                          | Exposed streams, ditches, irrigation channels     | <i>Anopheles, Culex</i>                                                                                                          |
|                          | Forest streams                                    |                                                                                                                                  |
|                          | Gravel stream beds                                |                                                                                                                                  |
| <b>Container habitat</b> | Tree-holes                                        | <i>Aedes, Anopheles, Culex, Orthopodomyia, Toxorhynchites</i>                                                                    |
|                          | Bamboo                                            | <i>Aedes, Anopheles, Eretmapodites, Uranotaenia</i>                                                                              |
|                          | Leaf axils, including bromeliads and plant bracts | <i>Aedes, Anopheles, Culex, Malaya, Mimomyia, Toxorhynchites</i>                                                                 |
|                          | Pitcher plants                                    | <i>Aedes, Anopheles, Culex, Mimomyia, Toxorhynchites</i>                                                                         |
|                          | Fallen fruit husks, Flower spathe                 | <i>Aedes, Culex, Eretmapodites, Toxorhynchites, Uranotaenia</i>                                                                  |
|                          | Fallen leaves                                     | <i>Aedes, Culex, Eretmapodites</i>                                                                                               |
|                          | Empty snail shells                                | <i>Aedes, Eretmapodites</i>                                                                                                      |
|                          | Rock-pools                                        | <i>Aedes, Anopheles, Culex, Toxorhynchites, Uranotaenia</i>                                                                      |
|                          | Crabholes                                         | <i>Aedes, Culex, Culiseta, Uranotaenia</i>                                                                                       |
|                          | Water tanks, cisterns                             | <i>Aedes, Anopheles, Culex, Culiseta, Culex</i>                                                                                  |
|                          | Latrines, septic tanks                            | <i>Culex</i>                                                                                                                     |
|                          | Domestic water-storage pots                       | <i>Aedes, Culex, Toxorhynchites, Eretmapodites</i>                                                                               |
|                          | Discarded tin cans, bottles, tyres                |                                                                                                                                  |

### 1.3.3. Adult mosquito ecology and behaviour

#### 1.3.3.1. Sugar foraging habits

Sugar is the primary energy resource for both female and male mosquitoes, and it is obtained in the form of fluids produced by plants or plant components, including nectar, honeydew, fruit juices and sap (Barredo & DeGennaro, 2020; Foster & Takken, 2004; Peach & Gries, 2020; Stone & Foster, 2013). Both male and female mosquitoes take sugar meals during their life. However, the frequency of sugar feeding is usually a function of sex, gonotrophic cycle and sugar resource availability (Gary Jr & Foster, 2006; Gouagna et al., 2010). In anautogenous species, sugar-feeding can continue for several days or weeks, if access to blood sources is limited (Stone & Foster, 2013). Mosquitoes are known to feed on several plant species, more remarkably flowering plants of family Apocynaceae (e.g., *Asclepias syriaca*), Fabaceae (e.g., *Acacia albida*, *Delonix regia*), Balsaminaceae (e.g., *Impatiens walleriana*), Anacardiaceae (e.g., *Mangifera indica*), Oleaceae (e.g., *Ligustrum quihoui*), and Caprifoliaceae (e.g., *Abelia chinensis*) (Barredo & DeGennaro, 2020; Peach & Gries, 2020), indicating that sugar-feeding is an important component of mosquito ecology and it needs to be fully harnessed for development of mosquito surveillance tools and novel control measures, such as attractive toxic sugar baits (Fraser et al., 2021; Muller et al., 2010). However, the approach has raised concerns due to potential negative impacts on non-target insects (Khallaayoune et al., 2013). Therefore, optimization is needed for these approaches to become more species-specific (Barredo & DeGennaro, 2020). The implications of sugar foraging in MBD transmission are poorly known. However, studies suggest that it may impact vectorial capacity (Stone & Foster, 2013). However, the design of innovative mosquito vector surveillance and control tools will certainly benefit from thorough understanding of mosquito sugar feeding ecology.

#### 1.3.3.2. Blood foraging habits and hosts preference

Blood meals are critical for female reproduction, specifically egg development, and also as a source of energy (Gary & Foster, 2001; Klowden, 1995; Stone & Foster, 2013). Mosquito blood-feeding is structured in a sense that blood meals are frequently taken from a group of certain animal hosts in preference over others (Burkot, 1988). Existing evidence indicates that mosquitoes obtain blood from a variety of animals, with strong inclination for warm-blooded vertebrates such as mammals and birds (Fikrig & Harrington, 2021; Snow & Boreham, 1979; Snyman, 2021; Weitz, 1960). The repertoire of cold-blooded hosts includes, but may not be

limited to, reptiles (snakes, turtles), amphibians (toads, frogs), fishes, earthworms, leeches (Chathuranga et al., 2018; de Silva et al., 2020; Fikrig & Harrington, 2021; Mary et al., 2013; Snyman, 2021; Tuten et al., 2012), and other insect larvae (nymphal cicadas, Lepidoptera larva) (Harris et al., 1969; Hribar, 2014; Martel et al., 2011). Few vector species frequently bite humans (e.g., *Ae. aegypti*, *An. funestus*, *An. gambiae* s.s) and are said to be anthropophilic, whereas those feeding predominantly on other animals, such as *An. implexus* (McCrae et al., 1976) are considered zoophilic. Species most inclined to feed on birds, such as *Coq. metallica*, *Coq. aurites* and *Cx pipiens* (Osorio et al., 2012; Service, 1990), are considered ornithophilic, while a fraction that feed almost exclusively on mammals are said to be mammalophilic (Fikrig & Harrington, 2021). Some species, such as *An. funestus* s.s, are specialists, feeding almost exclusively on a single or narrower range of hosts even in the presence of other hosts (Cohuet et al., 2004; Degefa et al., 2017; Harrington et al., 2001). In contrast, generalist species, such as *Ma. fuscopennata*, will obtain blood meals from a variety of hosts, viz., human, hippopotamus, frog, reptile, monkey, cattle, cat, rabbit, horse, and elephant (Mary et al., 2013; McClelland & Weitz, 1960). Clearly, generalist species may play an important role in maintaining zoonotic circulation of pathogens between humans and other susceptible animal hosts. Therefore, strong understanding of host-selection patterns by vector species is relevant for determining transmission dynamics, predicting the types of pathogens that vector and host animals may be exposed to, and eventually detecting and containing emergent mosquito-borne zoonotic diseases. Although host-preference is influenced by mosquito innate genetic basis (Main et al., 2016), no mosquito species is absolutely specific in its blood-feeding preferences. Factors such as host availability, abundance and accessibility to the hosts by vectors also play key roles in determining the magnitude of vector-host interactions (Takken & Verhulst, 2013). Generally, vectors will feed on hosts that are readily accessible and abundant (Burkot, 1988; Chaves et al., 2010).

#### **1.3.3.3. Blood foraging activity and resting habits**

The blood feeding activity and resting habits of mosquitoes are important indicators of their potential vector status and role in disease transmission (Smith & Ellis McKenzie, 2004). The two traits also have profound impacts on the design of surveillance systems and selection of vector control tools (Rozendaal, 1997). Mosquito foraging activity and resting habits are diverse, with remarkable spatiotemporal variability between species and within species groups or complexes (Haddow, 1954b). Many mosquito species are most active at night (Haddow,

1954b; Service, 1993), while others, including several *Aedes* species, feed only during daylight and at dusk (De Meillon, 1951; Haddow, 1964). A handful of species, notably *Er. chrysogaster*, bite indiscriminately at any time of the day or night (Haddow, 1956). In addition, some species (e.g., *Ae. africanus*, *Ae. aegypti*) will bite profusely at dawn and dusk, despite being active at any part of the day (Muhammad et al., 2020). Some anthropophagic or zoophagic species tend to enter human dwellings or animal shelters, respectively, to blood-feed and are termed endophagic, whereas others referred to as exophagic never blood-feed indoors (Gillies, 1956). Some mosquitoes referred to as endophilic will stay indoors during the entire period required for blood digestion and maturation of the ovaries (Cohuet et al., 2004; Degefa et al., 2017; Harrington et al., 2001). In contrast, a group of species will feed opportunistically both indoors and outdoors but will rest outdoors after having blood fed and are termed exophilic (Gillies, 1956; Tirados et al., 2006; Tuno et al., 2010).

The control of mosquito-borne disease transmission by bed nets and indoor residual spraying relies fundamentally on the propensity of vector species to rest indoors and to bite when a great proportion of the host population is asleep inside (Mathews, 2011; Rozendaal, 1997; van Emden & Service, 2004). Therefore, a change in host-seeking activity and resting habits of vector species will have a dramatic impact on the effectiveness of any control measures (Gatton et al., 2013; Milali et al., 2017). However, the degree of endophagy/exophagy, and endophily/exophily is relative, in a sense that vector species will adjust their habits so as to conform to behavioural patterns and sleeping habits of human hosts, and as well to avoid the effects of control measures (Gatton et al., 2013). For instance, some studies have reported increased exophagy of malaria vectors in the evening before bedtime (Finda et al., 2019; Rodríguez-Rodríguez et al., 2021). There is also evidence of induced exophagy following massive scale-up of indoor residual spraying and bed net distribution (Meyers et al., 2016; Moiroux et al., 2014; Moiroux et al., 2012; Taylor, 1975b). Host-seeking activity is also a product of variation of an assortment of poorly understood microclimatic and environmental conditions, notably air temperature, relative humidity, luminosity, and wind speed. In general, high mosquito flight activity occurs at relatively low average temperatures (17°C - 30°C), high humidity ( $\geq 70\%$ ), low wind speed ( $< 1$  m/s) and during the full moon (Bidleingmayer, 1967; Haddow, 1964; Kampango et al., 2011; Service, 1980).

#### 1.3.3.4. Dispersal habits: needles on the move

Mosquitoes disperse periodically in search of hosts and sugar sources, mates, and oviposition and larval habitats (Garrett-Jones, 1950; 1956; Provost, 1952; Service, 1997; Verdonshot & Besse-Lototskaya, 2014). Since the discovery of the role played by mosquitoes in transmission of malaria around the year 1905, information on the distance at which mosquitoes fly has been of interest due to implications for both disease propagation and area-wide implementation of control interventions. For instance, dispersal of uninfected vector species to locations beyond their geographic range can increase the risk of transmission in other localities, and also wider dispersal of infectious vectors can contribute to the spread of endemic or novel pathogens (Wilkerson et al., 2021), eventually creating new transmission foci or hotspots (Bousema et al., 2012; Marcantonio et al., 2016; Service, 1997). Therefore, knowing the dispersal habits of targeted vector species is relevant for the design of control strategies as it delimits the effective radius for deployment of surveillance tools and for vector control interventions (Silver, 2008). Furthermore, the ability of mosquitoes to disperse to wide distances is one of the key conditions for efficacy of control approaches involving transfer of genetic traits, such as the release of transgenic mosquitoes (Reiter, 2007).

The average maximum flight distance of mosquitoes is species (or taxon) specific. The average dispersal distance ranges usually from 50 metres to 50 kilometres (Verdonshot & Besse-Lototskaya, 2014). Some species such as *Ae. aegypti* and *Ae. africanus*, have weak dispersal capacity and seldom fly long distances (Guindo-Coulibaly et al., 2019; Liew & Curtis, 2004). The average maximum dispersal range of *Ae. aegypti* is between 224 - 240 metres (Marcantonio et al., 2019) and it is nearly 200 metres for *Ae. africanus* (Guindo-Coulibaly et al., 2019). However, the distance to which mosquitoes will disperse is influenced by the location of suitable hosts from oviposition sites and *vice versa*. Therefore, vector species adapted to domestic environments and urban areas such as *Ae. aegypti* (Harrington et al., 2001; Rose et al., 2020) are unlikely to disperse far from urbanized or human inhabited areas. In contrast, some mark-release-recapture experiments showed that some strong flying species such as most *Anopheles*, *Culex*, *Mansonia* and some *Aedes* species can fly as far as 2 km or sometimes up to 48 km away from release points (Gillies, 1961; Hamer et al., 2014; Verdonshot & Besse-Lototskaya, 2014). Notably, some authors have argued that it seems less likely a mosquito would engage in active appetitive long-range flight (e.g., 48 km). This type of long flight may be a passive migration assisted by prevailing winds (Garrett-Jones, 1956;

Service, 1980; 1997) or, sometimes, by means of transportation (Kennedy & Marjorie, 1999; Smith & Carter, 1984). Regarding this argument, there are reports of the appearance of *An. pharoensis* in an army camp in the Egyptian desert, at a distance of 72 km from the nearest possible breeding site after strong winds blowing north-east (Garrett-Jones, 1950). There is also evidence of resurgence of malaria in the coastal region of Israel after eradication. Investigations showed that reintroduction of infected *An. pharoensis* transported 280 km in prevailing south westerly winds was the main cause (Garrett-Jones, 1962). There is also direct evidence of adult *Ae. albopictus* being passively dispersed throughout Spain by cars (Eritja et al., 2017). For some vectors, long distance passive dispersal also occurs during immature stages (i.e., eggs and larvae). For instance, several *Aedes* species produce desiccation-resistant eggs enabling them to survive for months without water. This evolved trait has allowed vector species, particularly *Ae. aegypti* and *Ae. albopictus*, to be inadvertently transported and globally dispersed as dried eggs on automobile tyres, ornamental flowers, ships, boats, etc., into other regions outside their native distributional ranges (Cornel & Hunt, 1991; Kennedy & Marjorie, 1999; Lounibos, 2002; Reiter & Darsie, 1984).

#### **1.4. Mosquito taxa of relevance for public health**

Fewer than 150 mosquito species have been incriminated with transmission of pathogens that cause diseases or deformities to humans, livestock and animal wildlife (Harbach, 2007; Wilkerson et al., 2021). The majority of vector species are confined to the genera *Anopheles*, *Culex* and *Aedes* (Wilkerson et al., 2021). A *précis* on the ecological and distributional aspects of mosquito fauna of the three genera considered of great concern in public health, with emphasis on species found in the Afrotropical region and emergent species with the potential to become serious threats worldwide is presented below.

##### **1.4.1. The *Anopheles* fauna**

The subfamily Anophelinae comprises the genera *Anopheles*, *Bironelia* and *Chagazia* (Harbach, 2007; Service, 1993; Wilkerson et al., 2021). The genus *Anopheles* is distributed worldwide (Fig. 1.1), only being absent in polar regions and on remote islands in the Pacific and Atlantic oceans such as Micronesia, Polynesia, Fiji and New Zealand (Service, 1993; Wilkerson et al., 2021). In contrast, the genera *Bironelia* and *Chagazia* are restricted to the Australasia and Neotropical zoogeographical regions, respectively (Harbach, 2007). A total of 483 species, and more than 50 unnamed members of species complexes, are formally

recognized worldwide (Harbach, 2013). However, only about 70 are human malaria vectors of which around 40 are important vectors (Harbach, 2013; Service, 1993). Only the genus *Anopheles* contains vector species, most of which are concentrated in the subgenus *Cellia* (Harbach, 2007). Human malaria *Plasmodium* species, notably *P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale* and *P. knowlesi* (zoonotic between humans and some primates) are undoubtedly the most important pathogens transmitted by *Anopheles* mosquitoes. However, *An. (Cel.) gambiae* s.s, *An. (Cel.) arabiensis* and *An. (Cel.) funestus* s.s are also important vectors of lymphatic filariasis *Wuchereria bancrofti* (Manguin et al., 2010) and several arboviruses (Nanfack Minkeu & Vernick, 2018). Currently, the O'nyong-nyong virus is the only pathogenic arbovirus confirmed to be transmitted to humans by *An. gambiae* s.l and *An. funestus* in Africa. However, several other viruses with enormous potential to cause diseases in humans or other mammals have been isolated from *Anopheles* worldwide (Nanfack Minkeu & Vernick, 2018). Furthermore, recent evidence suggests that *An. gambiae* s.s may also be a vector of *Rickettsia felis*, a bacterium known to cause rickettsiosis, a malaria-like febrile disease in humans (Dieme et al., 2015; Socolovschi et al., 2012). Despite a global distribution, the relevance of *Anopheles* mosquitoes as disease vectors is currently restricted almost exclusively to the tropical and subtropical regions (Becker et al., 2020; Mouchet et al., 2004). Notorious *Anopheles* vectors of malaria in the Afrotropical region include *An. (Cel.) gambiae* s.s., *An. (Cel.) coluzzii*, *An. (Cel.) arabiensis*, *An. (Cel.) funestus*, *An. (Cel.) moucheti*, *An. (Cel.) nili* s.s, *An. (Cel.) ovengensis*, *An. (Cel.) pharoensis*, *An. (Cel.) merus*, *An. (Cel.) melas*, and *An. (Anopheles) coustani* (Fontenille & Lochouarn, 1999; Gillies & De Meillon, 1968; Irish et al., 2020; White, 1974). The dominant vectors in the region are *An. gambiae* s.s, *An. funestus* s.s, *An. arabiensis*, *An. (Cel.) nili* s.s, *An. moucheti*, *An. (Cel.) merus* and *An. (Cel.) melas* (Sinka, 2013; Sinka et al., 2010).

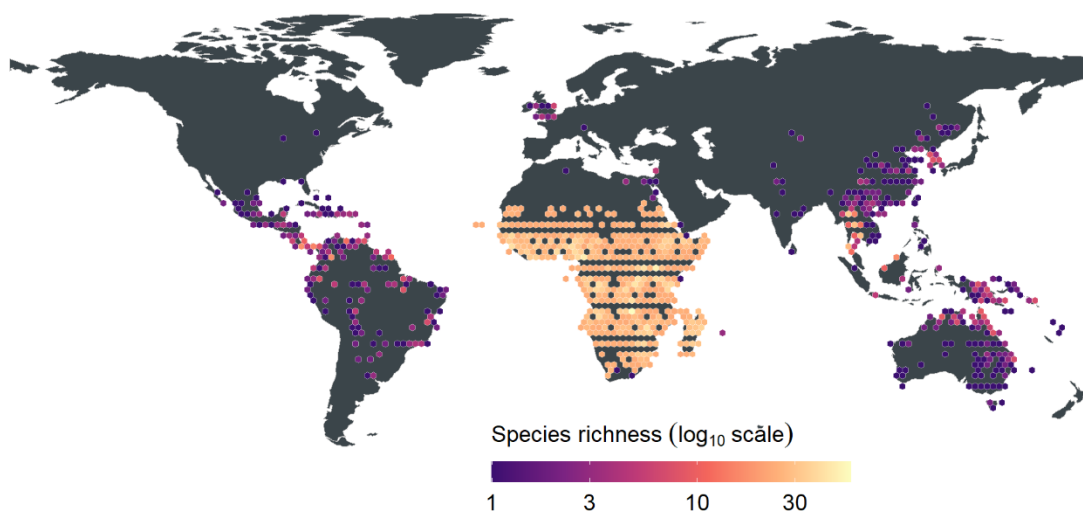


**Figure 1.1.** Global occurrence and distribution of *Anopheles* species richness. Map based on geocoded records obtained from the Mosquito Occurrence Dataset (Walter Reed Biosystematics Unit, 2021), African Vector Database (Kyalo et al., 2017), Mosquito Alert Dataset (Escobar & Južnič-Zonta, 2022), and mosquito occurrence data produced as part of this thesis. Land shapefile obtained from <https://www.naturalearthdata.com>.

*Anopheles* larvae develop in steady or slow-moving permanent and semipermanent water bodies exposed directly to sunlight and/or with emergent vegetation. Common larval habitats include swamps, marshes, rice fields and borrow pits, puddles, water-filled car tracks, ditches, drains and gullies. However, there is high phenotypic plasticity in larval habitat utilization. For instance, *An. (Cel.) arabiensis* and *An. (Cel.) gambiae* s.s also breed in highly polluted waters (Awolola et al., 2007; Azrag & Mohammed, 2018; De Silva & Marshall, 2012). The invasive urban Asian malaria vector *An. (Cel.) stephensi* breeds in artificial containers with clean water, such as domestic wells, plastic containers, cisterns, overhead water tanks, and roof gutters (Ishtiaq et al., 2021b). *Anopheles stephensi* has also been found in natural container habitats, such as river beds with pools, stagnant streams, slow foothill streams, temporary pools, and slowly moving water with and without vegetation (Ahmad et al., 2012). The ability of developing opportunistically in a wide range of natural and artificial containers has allowed rapid expansion of *An. stephensi* throughout urban settings (Ishtiaq et al., 2021a). The species, originating in the Southeast Asia and Middle East, has rapidly shifted its distributional range throughout the eastern Africa region, being initially reported in Djibouti, the Horn of Africa (Faulde et al., 2014), then in Ethiopia (Balkew et al., 2020), and most recently in Sudan (Ahmed

et al., 2021). There is increasing concern *An. stephensi* may soon expand southward and westward which could exacerbate the growing problem of emergent urban malaria in the sub-Saharan region (Takken & Lindsay, 2019).

Field observations in the Sahel region demonstrated that *An. gambiae* s.s., *An. coluzzii* and *An. arabiensis* can engage periodically in windborne high altitude (240 metres) migration over more than 150 kilometres (Dao et al., 2014; Huestis et al., 2019). This may somewhat explain invasion or recolonization of distant regions by less desiccation-tolerant mosquito taxa such as *Anopheles*.

Adult *Anopheles* are essentially nocturnal, biting earlier in the evening or later in the night. Biting activity can occur frequently indoors [e.g., *An. (Cel.) gambiae* s.s., *An. (Cel.) funestus* s.s., *A. (Cel.) moucheti* s.s.], outdoors [e.g., *An. (Cel.) pharoensis*, *An. (Cel.) merus*, *An. melas*] or with the same intensity in both environments [*An. (Cel.) nili* s.s and *An. (Cel.) arabiensis*] (Fontenille & Lochouart, 1999). Consistent broad daylight biting activity has been observed with *An. (Cel.) funestus* in Senegal, possibly as a response to selection by extended use of long-lasting insecticidal nets (LLIN) (Sougoufara et al., 2014).

#### 1.4.2. The *Aedes* fauna

The genus *Aedes* is the largest genus in the tribe Aedini, comprising a total of 935 species, divided into 75 subgenera (Harbach, 2013). The genus has a worldwide distribution (Fig. 1.2) with higher species richness being concentrated in the Afrotropical, Neotropical and Australasia regions (Harbach, 2007; Huang, 1972; 1979; 2004; Reinert & Harbach, 2005; Rueda, 2008). The subgenus *Stegomyia* is the most diverse in the *Aedes* genus, with a total of 175 species, including the most important vector species from the standpoint of transmission of pathogens and parasites (Harbach, 2007; Huang, 2004). The group includes several vectors of arbovirus and human filariasis, such as *Ae. (Stegomyia) aegypti* and *Ae. (Stg.) albopictus* (Service, 1993; Wilkerson et al., 2015; Wilkerson et al., 2021). These are the most widespread species, within and outside their native ranges in Africa for *Ae. (Stg.) aegypti*, and southeast (SE) Asia as well as the Indian Oceans Islands for *Ae. (Stg.) albopictus* (Bonizzoni et al., 2013; Soghigian et al., 2020). Currently, these two invasive species occur in virtually all continents, introduced via commercial activities (Lounibos, 2002; Powell, 2016; Wilkerson et al., 2021). Some vector species of global and regional importance, with emphasis on the Afrotropical region, include *Ae. (Stg.) aegypti*, *Ae. (Stg.) albopictus*, *Ae. (Stg.) africanus*, *Ae. (Stg.)*

*metallicus*, *Ae. (Stg.) bromeliae*, *Ae. (St.) simpsoni* s.s, *Ae. (Stg.) luteocephalus*, *Ae. (Aedimorphus) vittatu*, *Ae. (Neomelaniconion) mcintoshii*, *Aedes (Neo.) circumluteolus*, *Ae. (Dic.) furcifer*, *Aedes (Dic.) taylori*, *Ae. (Stg.) polynesiensis*, and *Ae. (Stg.) scutellaris* (Huang, 2004; Wilkerson et al., 2015; Wilkerson et al., 2021).

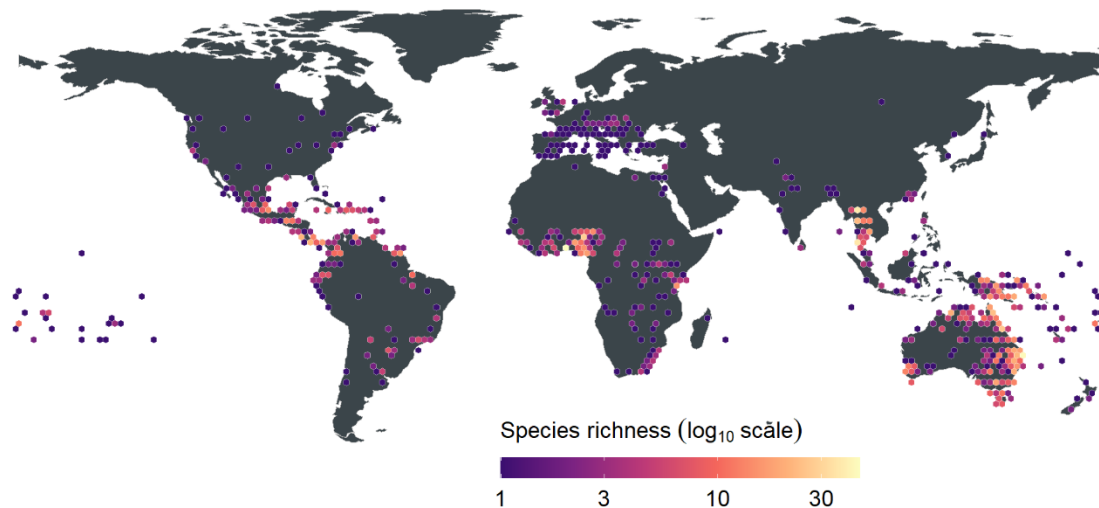


**Figure 1.2.** Global occurrence and distribution of *Aedes* species richness. Map based on geocoded occurrence records obtained from the Mosquito Occurrence Dataset (Walter Reed Biosystematics Unit, 2021), African Vector Database (Kyalo et al., 2017), Mosquito Alert Dataset (Escobar & Južnič-Zonta, 2022), and mosquito occurrence data produced as part of this thesis. Land shapefile obtained from <https://www.natureearthdata.com>.

Infectious agents known to be transmitted to humans by the *Aedes* mosquitoes includes several viruses (Wilkerson et al., 2021), most notably DENV, YFV, CHIKV, ZIKV, Rift Valley fever virus (RVFV) (Berger, 1975; Wilkerson et al., 2015; Wilkerson et al., 2021), and human lymphatic filariasis *Brugia malayi* and *Wuchereria bancrofti*. *Aedes*-borne lymphatic filariasis is restricted to the South Pacific Islands where it has been maintained by *Ae. (Stg.) polynesiensis* and *Ae. (Stg.) scutellaris*, (Belkin, 1962; Burkot & Ichimori, 2002). The presence of *Ae. (Stg.) albopictus* has been confirmed in some sub-Saharan region countries (Cornel & Hunt, 1991; Fontenille & Toto, 2001; Kampango & Abilio, 2016; Ngoagouni et al., 2015; Toto et al., 2003). However, its vector status has yet to be delineated.

Immatures of *Aedes* develop in a wide range of natural and artificial containers, moist substrates, or on soil that is temporarily inundated or subject to a rising water levels (Becker et al., 2020). In general, larval habitats usually include collections of water such as flooded meadows, small ponds, rock-pools, leaf axils, tree-holes, pottery, coconut and mollusc shells, fallen leaves and flower spathes, bamboo and a myriad of domestic and discarded containers, such as plastic bottles, plastic drums, overhead or ground water tanks, roof gutter, tin cans, tyres, etc., (Abilio et al., 2018; Burkot et al., 2007; Saleh et al., 2018; Zahouli et al., 2017). Most species produce desiccation-resistant eggs capable of remaining viable for months (Huang, 1972; Huang, 1997; Huang, 2004; Rezende et al., 2008). Several disease vectors are diurnal, exophagic, exophilic, anthropophagic or zoophagic or opportunistic. A notable exception is *Ae. (St.) aegypti*, which is strongly anthropophagic and endophagic (Harrington et al., 2001). Most species bite at any time of the day, but with high intensity at dusk or dawn (Haddow, 1954a). *Aedes (St.) aegypti* and *Ae. (St.) albopictus* have bimodal diurnal biting activities with peaks around dawn and dusk (Christophers, 1960; Hawley, 1988; Muhammad et al., 2020).

#### 1.4.3. The *Culex* fauna

The tribe Culicini comprises a total of 807 species assigned to four genera viz., *Culex*, *Deinocerites*, *Galindomyia* and *Lutzia*. The genus *Culex* is by far the largest and most common and includes most of the medically important and pest species, confined in the subgenus *Culex* (Harbach, 2013; Jupp, 1986). *Culex* mosquitoes are widely distributed in all zoogeographical regions (Fig. 1.3), except the western Palaearctic and Nearctic Regions (Becker et al., 2020; Harbach, 2013). Major vector species include members of the *Cx. (Cux.) pipiens* complex, notably *Cx. (Cux.) pipiens* s.s and *Cx. (Cux.) quinquefasciatus*, the primary vectors of West Nile virus (WNV) worldwide (Ciota, 2017). Species such as *Culex (Cux.) univittatus*, *Cx. (Cux.) neavei*, *Cx. (Cux.) perexiguus*, *Cx. (Cux.) australicus*, *Cx. (Cux.) globcoxitus*, and *Cx. (Cux.) tarsalis* are WNV vectors of regional relevance elsewhere in Africa, Europe, Australia and the Americas (Ciota, 2017). *Culex (Cux.) quinquefasciatus* is also the primary and widely distributed vector of the filarial worm *W. bancrofti* (Subra, 1981; White, 1989). The rice field-associated, cosmopolitan species *Cx. (Cux.) tritaeniorhynchus*, together with *Cx. (Cux.) vishnui*, *Cx. (Cux.) fuscocephala* and *Cx. (Cux.) gelidus*, which are confined to the Australasia region, are major vectors of the highly lethal and deforming Japanese encephalitis virus (JEV) in Asia (Wada et al., 1970). In Africa, *Cx. (Cux.) theileri*, *Cx. (Cux.) neavei* and *Cx. (Cux.)*

*antennatus*, among many other *Culex* species, maintain and spread RVFV (Linthicum et al., 2016). Several *Culex* are also notorious nuisance pests (Harbach, 2013).

Development of the immature stages occurs primarily in semi-permanent or permanent water bodies. However, a fraction of species breeds exclusively in leaf axils, tree-hollows, bamboo stumps, small pools of water and ponds, marshes in rice fields, crab holes, and tyres. Some exploit artificial containers and water-filled discarded domestic utensils as well as groundwater such as in wells (Edwards, 1941; Harbach, 2013). Larvae of a few species, such as *Cx. quinquefasciatus* are commonly found in turbid and highly polluted groundwater bodies that contain decaying organic matter, including human wastes, such as ditches, drains, sewage, and dumping areas (Castro et al., 2010; Charlwood, 1994; Subra, 1981). Most adult females are vicious crepuscular and nocturnal exophagic and zoophagic mosquitoes, feeding frequently on other mammals and birds (Hocking, 1971). However, females of *Cx. quinquefasciatus* have adapted to anthropophagic habits, feeding frequently on humans both indoors and outdoors (Subra, 1981).

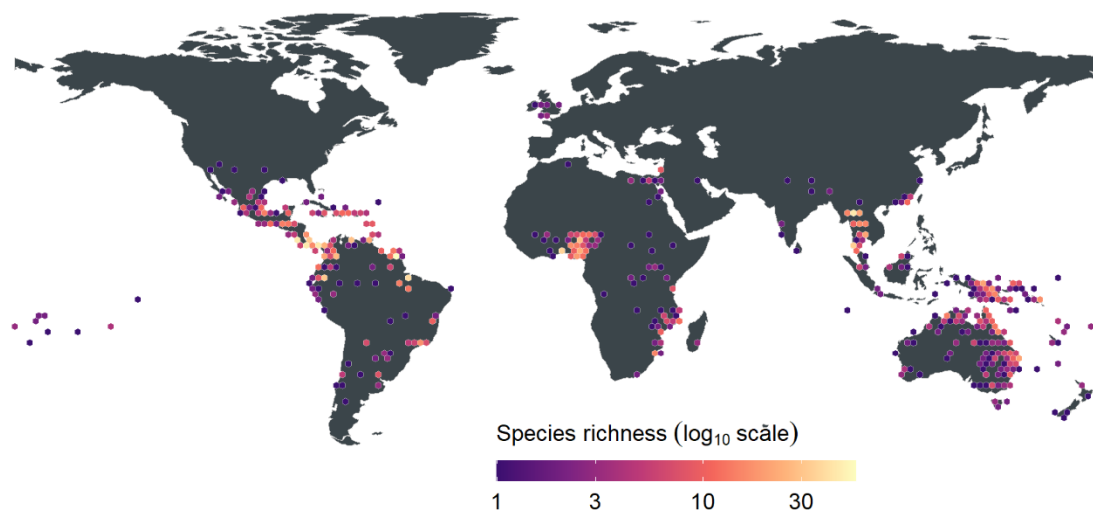


**Figure 1.3.** Global occurrence and distribution of *Culex* species richness. Map based on geocoded occurrence records obtained from the Mosquito Occurrence Dataset (Walter Reed Biosystematics Unit, 2021), African Vector Database (Kyalo et al., 2017), Mosquito Alert Dataset (Escobar & Južnič-Zonta, 2022), and mosquito occurrence data produced as part of this thesis. Land shapefile obtained from <https://www.natureearthdata.com>.

## 1.5. Mosquito control methods

The goal of interventions targeting mosquito vectors is to reduce the transmission of pathogens and incidence of associated diseases to a level at which they do not constitute a public health problem (Ferguson, 2018). As there is a general lack of safe and affordable vaccines with satisfactory preventive efficacy against major mosquito-borne pathogens, vector control remains the only cost-effective alternative for control and prevention of MBD transmission (Ferguson, 2018; Wilson et al., 2020). Control approaches comprise methods that target mosquitoes at immature (egg, larva instars and pupa) and adult stages (Fig 1.4). These are in turn divided into major categories by their mechanisms or modes of action, namely, whether they kill (e.g., space spraying, indoor residual spraying) or prevent mosquitoes contact with a potential host (e.g., bed nets and spatial repellents) (Achee et al., 2015). Traditional control measures involve use of natural or synthetic chemical insecticides, environmental actions and biological agents (Rozendaal, 1997). However, interest in innovative genetic control measures is growing due to the limitations of traditional approaches (Fig 1.4).

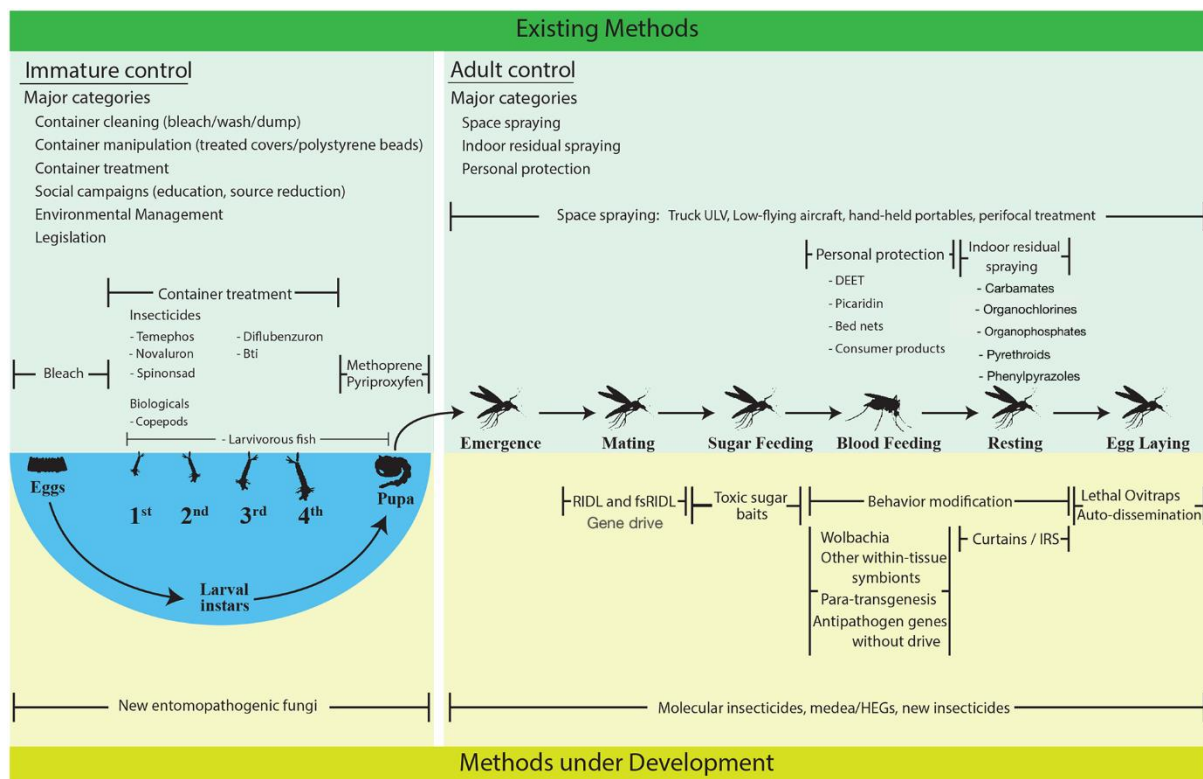


**Figure 1.4.** Mosquito control approaches divided by conventional and innovative methods that target larval and adult stages. Reproduced with slight modification from Achee et al. (2015).

Bti: *Bacillus thuringiensis israelensis*, CRISPR: clustered regularly interspaced short palindromic repeats; RIDL: release of insects carrying a dominant lethal gene; DEET: N, N-diethyl-meta-toluamide; ULV: ultra-low volume; IRS: indoor residual spraying.

### 1.5.1. Chemical control of larval stages

Before the advent and widespread use of synthetic insecticides, mosquito control entailed primarily targeted treatment of larval habitats with petroleum oils, plant-derived extracts, and the heavy metal salts “Paris Green”. Larval habitats were also covered with kerosene and diesel oil or other readily available oils (van Emden & Service, 2004). The oils work by changing the surface tension of the water which interferes with the respiration of mosquito larvae and pupae, resulting in suffocation. However, some compounds also cause intoxication (van Emden & Service, 2004). Plant extracts used comprised pyrethrum, from flower heads of *Chrysanthemum cinerariifolium*, rotenone (a root extract of the plant *Derris elliptica*) and nicotine (from the plant *Nicotiana tabacum*), and they act either on the nervous or respiratory system (van Emden & Service, 2004). Paris Green has toxic radicals of arsenic or fluorosilicate formulated as salts of lead or sodium. The radicals are poisonous to the digestive tract after being released on ingestion by an insect. Earlier larvicides also included formulations of the neurotoxic insecticide dichloro-diphenyl-trichloroethane (DDT). Due to long environmental persistence and concerns regarding harmful effects on the non-target organisms, oil extracts were replaced by synthetic and biological larvicides (van Emden & Service, 2004).

The main goal of larviciding is to reduce the density of vectors that would eventually become adults. These types of control practices can reduce the adult mosquito population and malaria transmission in urban settings. (Geissbuhler et al., 2009). However, in most situations, a successful larval control has only been achieved if targeted vector species utilize permanent or semi-permanent water bodies, which are relatively few, fixed and findable (Coosemans & Carnevale, 1995; Curtis & Townson, 1998; WHO, 2013). One important limitation of larviciding is the low residual persistence of larvicides, requiring repeated treatments in a short period of time. Synthetic larvicides currently approved for use in public health include the organophosphates temephos and pirimiphos-methyl and the benzoylurea diflubenzuron, as well as insect growth regulatory hormone mimics, notably pyriproxyfen, novaluron and methoprene (Water Sanitation Hygiene and Health, 2017)

### 1.5.2. Chemical control for adult stages: to kill or to repel?

Chemical control has become the core tool for management of adult mosquitoes worldwide (van Emden & Service, 2004). The invention of synthetic insecticides meant adult mosquito control interventions could be rapidly scaled up across wide ranges of ecological and epidemiological settings. Currently, the most notable, cost-effective, and widely used adult control measures for *Anopheles*, *Aedes* and *Culex* mosquito vectors are indoor residual spraying (IRS) and long-lasting insecticide treated bed nets (LLINs). Insecticide-treated nets are the most widespread intervention (Bhatt et al., 2015). The rationale for combining these two methods is that some anthropophilic mosquito vector species tend to feed and spend their gonotrophic cycle resting inside human dwellings or closed spaces, so there would be a high likelihood of vector species coming into contact with indoor, insecticide treated surfaces (Coosemans & Carnevale, 1995; Rozendaal, 1997). The goal of adult control measures is to reduce the density, survivorship and longevity of mosquitoes, and thereby, the probability of vector species to become infective (Coosemans & Carnevale, 1995). Insecticide impregnated bed nets offer extra protection as a barrier against mosquito bite exposure. In addition, LLINs also act as baited traps for hungry females that are attracted by the host sleeping under the treated material. Therefore, it is expected that, with LLINs, vectors are killed before biting their hosts (Coosemans & Carnevale, 1995; Rozendaal, 1997).

Over the last five decades, several insecticides have been developed for adult mosquito control but only a handful have received formal approval for use in public health. These comprise mostly organochlorine, organophosphate, carbamate and pyrethroid insecticides (Hemingway & Ranson, 2005; WHO, 2006). Pyrethroids are the most widely used class of insecticides in public health (WHO, 2022). However, despite their central role in the fight against MBDs, it has become clear that both IRS and LLINs only offer protection against a fraction of indoor MBD transmission. Moreover, in some regions, outdoor transmission can be as high as indoors (Keita et al., 2021).

Several personal protection approaches have been developed to control outdoor residual MBD transmission not targeted by IRS and LLINs. Repellents are among the most widely used personal protection tools against insect bites (Riffell, 2019; Rozendaal, 1997). Repellents are divided into plant-based oils and synthetic chemicals (Debboun et al., 2015). They are applied directly to the skin or to clothing, and other fabrics, or vaporized and sprayed into the environment (Debboun et al., 2015; Rozendaal, 1997). Chemical repellents such as DEET

(N,N-diethyl-3- methylbenzamide), Picaridin (2-(2-hydroxyethyl)-1-piperidine-carboxylic acid 1-methylpropyl -ester), and IR3535 (3-[N-butyl – N-acetyl] – amino-propionic acid ethyl ester) are the most widely studied and commercialized (Debboun et al., 2015). DEET is considered the gold-standard of insect repellents (Leal, 2014), being effective against all groups of biting arthropods and even leeches (Goodyer et al., 2010; White & Moore, 2015). Another group of chemical repellents that has gained global acceptance for use in open spaces are the pyrethroids allethrin, metofluthrin, and transfluthrin (White & Moore, 2015). An increasing number of field studies have reported a significant reduction in human exposure to several mosquito vector species in the presence of metofluthrin and transfluthrin (Lucas et al., 2007; Mmbando et al., 2018; Morrison et al., 2022; Ogoma et al., 2017).

#### **1.5.2.1. The emergence of insecticide resistance as a challenge to using chemical control**

Despite the global success achieved against MBDs by means of chemical control interventions, the sustainability of these gains is under permanent threat due to rapid expansion of resistance against the nine classes of chemical insecticides used in vector control products prequalified or approved for public health use (Mota-Sanchez & Wise, 2019). Thus, the development of resistance in the three major mosquito genera *Anopheles*, *Aedes* and *Culex* complicates even more the effectiveness of the vector control interventions given the limited insecticide options available for diverse vector populations (Georghiou, 1990; Liu, 2015). Insecticide resistance is broadly defined as the ability of a mosquito to survive exposure to a standard dose of insecticide that has proven to be lethal to a susceptible population (WHO, 2022). Insecticide resistance may result from preadaptation in the sense that there already exist rare individuals in the population that have never been exposed to the stressor but intrinsically carry one or more resistance alleles. Such polymorphisms or increased expression of the mutant alleles allow mutant insects to survive exposure to the insecticide (Curtis et al., 1978; Georghiou & Taylor, 1977; Hemingway & Ranson, 2000; IRAC, 2011; Liu, 2015).

Intense insecticide use often constitutes a constant pressure that favours insects carrying resistance alleles to survive and pass the trait on to their offspring. As such, the rare and resistant mosquitoes in a population continue to multiply and become the dominant phenotype while the susceptible mosquito populations are reduced in numbers and may eventually be eradicated (Georghiou, 1994; Hemingway & Ranson, 2000; IRAC, 2011; Liu, 2015). How quickly resistance develops depends on several factors, including the presence and frequency of resistant genes, insect reproduction rate (number of offspring per generation), the migration

and host range, the persistence and specificity of the insecticidal products in the environment, and the rate, timing and number of applications (Curtis et al., 1978; Georghiou, 1994; Georghiou & Taylor, 1977). The fitness costs associated with inherited mutations may also prevent or facilitate resistance expansion (Brown et al., 2013; Diniz et al., 2015; Freeman et al., 2021; Hanai et al., 2018; Nkahe et al., 2020; Shi et al., 2004). Resistant populations evolve faster if the resistance gene is dominant, and slower if it is recessive (Curtis et al., 1978; Georghiou & Taylor, 1977). However, the expression of dominance depends on the insecticide dose applied (Curtis et al., 1978).

The need for effective strategies in resistance management is becoming more pressing as the number of insecticide resistant species continues to increase worldwide while insecticide resources are diminishing (Georghiou, 1994). Therefore, understanding the ecological and evolutionary drivers of insecticide selection pressure is vital to design and implement measures to prevent or delay the development of insecticide resistance in mosquito populations. The mechanisms by which insects circumvent the lethal action of insecticides are divided into metabolic, target site, cuticular and behavioural resistances (Fig. 1.5) (Hemingway & Ranson, 2000; Liu, 2015; WHO, 2022). These mechanisms are not mutually exclusive, with different mechanisms possibly present at the same time. For instance, a recent study found that insecticide resistance in a *Cx. quinquefasciatus* population of Yaoundé, Cameroon, was associated with the presence of both metabolic and target site insensitivity resistance mechanisms, which conferred resistance against bendiocarb, malathion, deltamethrin and permethrin (Talipouo et al., 2021). Similar multiple resistance mechanisms had previously been observed in *An. gambiae* and *Cx. quinquefasciatus* from Benin (Corbel et al., 2007).

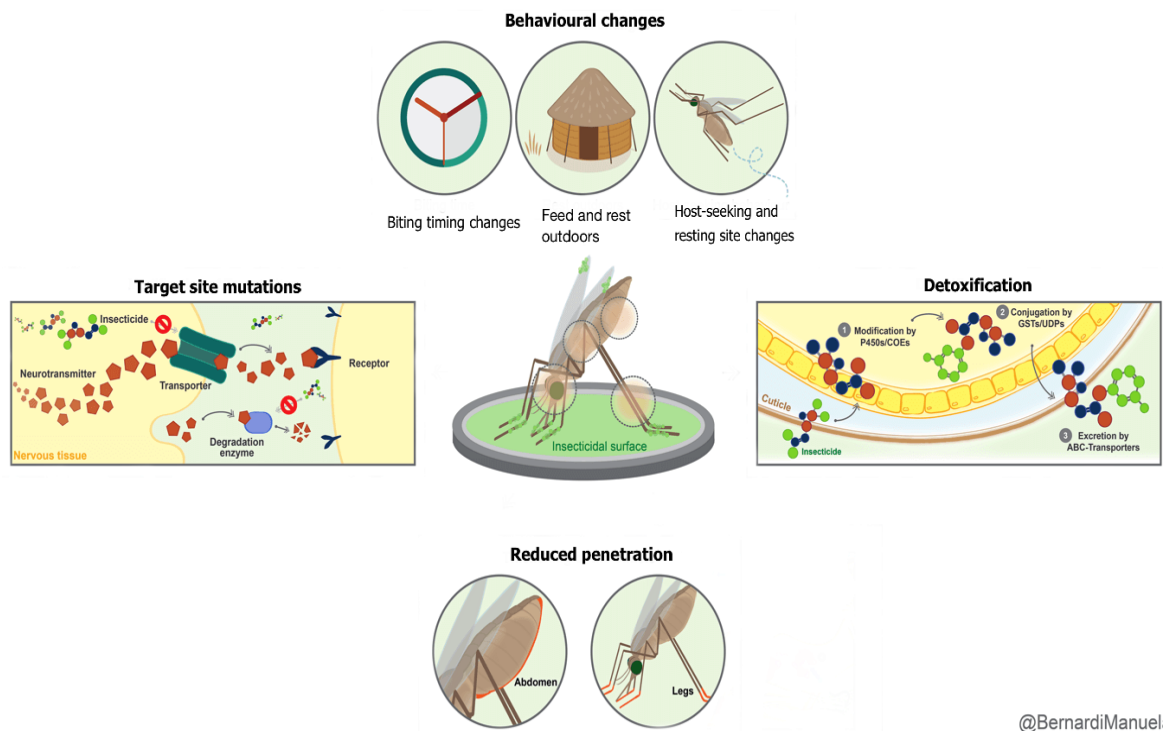


**Figure 1.5.** Representation of four known types of insecticide resistance mechanisms. Resistance may be selected at different sites in the insect. The insect cuticle may be selected for lower permeability, new or more abundant metabolic enzymes may be selected for sequestration and/or break down the insecticide more efficiently; and altered target sites may be selected on so that the insecticide no longer binds (Georghiou, 1994; Liu, 2015). Some endophagic/endophilic insects may shift their feeding habits, displaying a predominantly exophagic/exophilic tendency. Diagram modified from <https://tinyurl.com/y3vhds4k>.

Metabolic resistance is the most common mechanism of insecticide resistance found in mosquitoes (IRAC, 2011). It involves overproduction of detoxification enzymes through amplification of the detoxification genes or overexpression of these same genes. Some detoxification enzymes prevent the insecticide from reaching its target site within the mosquito, whereas others protect the mosquito from toxic insecticide metabolites (Hemingway & Ranson, 2000; Liu, 2015; WHO, 2022). Three major enzyme families are associated with metabolic resistances, viz., cytochrome P450 monooxygenases (P450s), carboxylesterases (COEs) and glutathione S-transferases (GSTs) (Table 1.2) (Georghiou, 1994; Hemingway & Ranson, 2000; Liu, 2015). The P450s comprises the largest family and are associated with metabolic resistance to virtually all classes of insecticides approved for public health, but specially for pyrethroids and neonicotinoids (Table 1.2). The GSTs are involved in metabolic detoxification and

excretion of organochlorines, and to a lesser extent, organophosphates and pyrethroids. The COEs confer resistance to organophosphates, carbamates, and to a lesser extent, pyrethroids (Table 1.2) (Hemingway & Ranson, 2000; Liu, 2015). The second most dominant type of resistance mechanism in insects is the presence of target site mutations (IRAC, 2011). This is the result mutations (point mutations) in genes encoding target proteins, usually in the neuromuscular system (Fig. 1.5), so that the insecticide can no longer effectively bind to the intended target site. Consequently, the insect is either unaffected or is less affected by the insecticide (Hemingway & Ranson, 2000; Liu, 2015). Target site mutations are associated with resistance to organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, phenylpyrazole, avermectines and juvenile hormone mimics (Table 1.2) (WHO, 2022). Resistance to DDT and pyrethroids occurs due to point mutations in the gene encoding the transmembrane voltage-gated sodium channel (VGSC) receptors in nervous and muscular cells, conferring so-called knockdown (*kdr*) gene mutation resistance (Agence Nationale de Sécurité Sanitaire, 2021; Liu, 2015). For organophosphates and carbamates, the mutations occur in the gene (*ace-1*), which encodes for the post-synaptic acetylcholinesterase (AChE) receptor (Agence Nationale de Sécurité Sanitaire, 2021; Liu, 2015). For dieldrin and fipronil, the mutations occurs in the gamma-aminobutyric acid (GABA) receptor gene (*rdl*), whereas for neonicotinoids, mutations occur in the gene (*N/a*) encoding a nicotinic acetylcholine receptor subunit (Table 1.2) (WHO, 2022).

**Table 1.2.** Insecticides used for mosquito vector control and associated resistance mechanisms.  
Table adapted from Agence Nationale de Sécurité Sanitaire (2021) and WHO (2022).

| Insecticide class               | Biochemical targets                               |      |               | Resistance mechanisms |                              |              |            |                      |                                                              |     |                     |      |
|---------------------------------|---------------------------------------------------|------|---------------|-----------------------|------------------------------|--------------|------------|----------------------|--------------------------------------------------------------|-----|---------------------|------|
|                                 |                                                   |      |               | Target site mutations |                              |              |            | Metabolic mechanisms |                                                              |     | Reduced penetration |      |
|                                 | VGSC                                              | AChE | GABA receptor | ACh receptor          | <i>kdr</i>                   | <i>ace-1</i> | <i>rdl</i> | <i>Na/a</i>          | COE                                                          | GST |                     | P450 |
| Organochlorines                 | X                                                 |      |               |                       | ++                           |              |            |                      | ++                                                           | +   |                     | +    |
| Cyclodiens                      |                                                   |      | X             |                       |                              | ++           | ++         |                      |                                                              |     | +                   | +    |
| Organophosphates                |                                                   | X    |               |                       |                              | ++           |            |                      | ++                                                           | +   | +                   | +    |
| Carbamates                      |                                                   | X    |               |                       |                              |              |            |                      |                                                              |     | +                   | +    |
| Neonicotinoids                  |                                                   |      |               | X                     |                              |              |            | +                    | ++                                                           |     | ++                  | +    |
| Pyrethroids                     | X                                                 |      |               |                       | ++                           |              |            |                      | +                                                            | +   | ++                  | +    |
| Phenelpryrazole                 |                                                   |      | X             |                       |                              |              | ++         |                      |                                                              |     | +                   | +    |
| Avermectines                    |                                                   |      | X             |                       |                              | Unknown      |            |                      |                                                              |     | +                   | +    |
| Insect growth regulators (IGRs) | Hormone receptors and chitin biosynthetic pathway |      |               |                       | Target site mutations        |              |            |                      |                                                              |     | +                   | +    |
| Bti toxins                      | Intestinal wall receptors of mosquito larvae      |      |               |                       | Mutation of binding proteins |              |            |                      | Alteration of toxins, enhanced immunity, toxin sequestration |     |                     | +    |
| Bs toxin                        |                                                   |      |               | +                     |                              |              |            |                      |                                                              |     |                     |      |

*ace-1* - gene encoding acetylcholinesterase; VGSC - voltage-gated sodium channel, GABA - gamma- Aminobutyric acid; ACh - acetylcholine; AChE - acetylcholinesterase; COE: carboxylesterase; GST - glutathione S-transferase; P450 - cytochrome P450 monooxygenases; *kdr* - knockdown resistance gene; *rdl* - resistance to dieldrin gene; *Na/a* - nicotinic acetylcholine receptor subunit. X indicates the correspondence between insecticide family and biochemical target; +/++ indicates the strength of the association between the resistance mechanism and the phenotypic resistance to the insecticide class.

Target site resistance is also associated with resistance to some insecticides acting outside nervous system targets and muscular systems, such as, toxins produced by *Bacillus thuringiensis* (Bti) and *Bacillus sphaericus* (Bs), and several insect juvenal hormone mimics. Reports of field resistance to Bti and Bs resistance in mosquito populations are rare (Mota-Sanchez & Wise, 2019; Tetreau et al., 2013). However, tolerance to Bti and Bs crystalized toxins has been reported in the mosquitoes *Cx.pipiens* and *Cx quinquefasciatus* (Mota-Sanchez & Wise, 2019; Saleh et al., 2003). Despite this, an increasing body of evidence suggests that resistance to Bti is well established in agricultural pests (Jurat-Fuentes et al., 2021), with the most frequent resistance mechanisms involving mutations to binding proteins, namely, aminopeptidase-N, alkaline phosphatase, and cadherins, and as well alteration of the membrane protein ABC transporter (Tetreau et al., 2013). Other known mechanisms in agriculture insect pests against Bti and Bs toxins are enhanced immunity, toxin sequestration, and epithelial regeneration (Tetreau et al., 2013).

Cuticular resistance is attributed to reduced absorption of insecticide by a mosquito due to thickening or alteration in composition of the cuticle. Cuticular modifications are attributed to overexpression of one or more of the diverse genes involved in the formation of the insect cuticle (Balabanidou et al., 2019; Hemingway & Ranson, 2000; IRAC, 2011), and have been associated with resistance to insecticides in several vectors species, such as *An. gambiae* (Balabanidou et al., 2019) and *Ae. aegypti* (Borel et al., 2021a). In general, cuticular resistance can affect a broad range of insecticides and may be found in conjunction with metabolic and *kdr* resistance mechanisms (Georghiou, 1994).

In behavioural resistance, the insect adapts its behaviours such as feeding, biting, and resting habits to avoid contact with insecticides. Some vectors shift their host-seeking habits for blood-meals to earlier in the evening or later in the morning. The underlining genetic mechanism remains unknown (Gatton et al., 2013; WHO, 2022). One of the earliest observations of a change in feeding behaviour by the malaria vector *An. farauti* to the prolonged use of DDT for residual spraying in the Solomon Islands (1975a). Insecticide induced behavioural changes are increasingly reported in malaria vectors throughout the sub-Saharan region (Meyers et al., 2016; Moiroux et al., 2014; Moiroux et al., 2012; Sangbakembi-Ngounou et al., 2022; Sougoufara et al., 2014), representing a huge challenge to the prospect of eradication of malaria in Africa.

### 1.5.3. Biological control

Biological control involves the use of predators, parasites, pathogens, natural enemies, parasitoids, or toxins from other organisms to reduce the size of a target population (Hemingway, 2005). Thorough reviews of organisms used as biological agents for mosquito control are given elsewhere (Benelli et al., 2016; Kamareddine, 2012). Biological control has an advantage over conventional broad-spectrum insecticides for being species-specific with correspondingly little disruption of non-target organisms in the environment (Hemingway, 2005; Rozendaal, 1997). However, there is also the potential for resistance selection (Hemingway, 2005; Rozendaal, 1997). Biological control may be most effective when used in combination with environmental manipulation or the application of larvicides that do not harm the biological control agents (Rozendaal, 1997). The most frequently used organisms are larvivorous fish, entomopathogenic fungi, bacteria, viruses, nematodes, and aquatic plants (Kamareddine, 2012; WHO, 2006). Of these, larvivorous fish *Gambusia affinis* and neurotoxins from the bacteria *Bacillus thuringiensis* var. *israelensis* and *B. sphearicus* are the

most widely used. There is no consistent evidence linking MBDs transmission reduction after introduction of *G. affinis* (Walshe et al., 2013). However, some studies on the effect of *B. thuringiensis* toxins are promising (Dambach et al., 2020). Several studies have shown reduction of *Ae. aegypti* abundance in the presence of *G. affinis* and *B. thuringiensis* (Ahmad Zaki et al., 2020; Hustedt et al., 2021). Problematically, there have been reports of adverse effects on the structure of non-target insect and amphibian fauna (Kats & Ferrer, 2003; Poulin, 2011) that may threaten long-term use of *B. thuringiensis*, *G. affinis*, and probably also other biological control methods in some regions.

#### 1.5.4. Genetic control

Genetic methods for mosquito control were first proposed in the 1940s (Coleman & Alphey, 2004). The technology has expanded dramatically since then and, currently, genetic control tools have been divided into two broad categories: those that aim to suppress vector populations, and those that modify them to become refractory to pathogen transmission (Flores & O'Neill, 2018). The controlling mechanisms of these two approaches are genetic traits introduced into the wild population by mating (Coleman & Alphey, 2004). Currently, two most widely applied population reduction approach are the sterile insect technique (SIT), introduced in the 1950s (Knippling, 1959), and release of insects carrying dominant lethal genes (RIDL) (Flores & O'Neill, 2018). However, the SIT technique cannot be considered genetic approach, as it involves insects mass production, sterilization by radiation and subsequent release of sterile male in the target region (Helinski et al., 2008). Females inseminated by either sterile males or males carrying dominant lethality fail to produce viable offspring, leading to reduction of the population (Flores & O'Neill, 2018; Helinski et al., 2008). Contrarily, population modification involves release of both male and female mosquitoes carrying a heritable factor that reduces or blocks their ability to transmit the target pathogen (Flores & O'Neill, 2018). The factor will spread through the target vector population as modified mosquitoes mate with wild individuals, rendering the offspring incapable of transmitting the pathogen (Flores & O'Neill, 2018). Population modification approaches include the deployment of *Wolbachia*, a pathogen-blocking endosymbiotic bacteria, and gene-drive mechanisms, such as the CRISPR–Cas9 system.

### **1.5.5. Environmental measures for mosquito control**

Environmental measures to control mosquitoes date back more than a century, with the pioneering works by Watson (1911) in Malaysia. The author firstly demonstrated that targeted transformations of the environment around human settlements could reduce malaria incidence. The main purpose of environmental control measures is to reduce the abundance of vector populations (and eventually disease transmission) by making the environment either unsuitable or by intensifying the action of natural factors that prevent establishment, development, survival and contact between vectors and humans (van Emden & Service, 2004; WHO, 1982). However, environmental management is currently used on only a limited scale in endemic countries, despite largely proven effectiveness in the control and elimination of targeted vector species (Takken et al., 1990).

Environmental management practices have been classified into environmental modification and environmental manipulation. Environmental modification consists of planned permanent or long-lasting transformation of land, water and vegetation associated with target mosquito populations (WHO, 1982). Some examples of environmental modification include drainage or filling of large water channels and collections, closing, screening, or covering larval habitats, impoundment of water body margins, etc. Once established, environmental modification can remain effective indefinitely, requiring only adequate maintenance (WHO, 1982). On the other hand, environmental manipulation aims at producing temporary conditions unsuitable for vector survivorship (WHO, 1982). Some of the common environmental manipulation interventions includes, removal of vegetation, fluctuation of water levels, stream flushing, shading, straightening and steepening of shorelines, changing water salinity, and removal and proper treatment of wastes (WHO, 1982; 2013). Therefore, unlike environmental modification, environmental manipulation must be repeated to retain efficacy and it is normally directed at few or one particular vector species (WHO, 1982; 2013). Modification and/or manipulation of human habitation structure are also considered environmental control interventions. Habitat modification by covering eaves, and installation of insect screens over windows, doors, and walls are common house modification interventions to prevent or reduce contact with vectors (Ogoma et al., 2010). Raising house on stilts is also another type of house modification strategy showed to reduce mosquito density inside human houses (Charlwood et al., 2003). Interventions aimed at changing human behaviour conducive to a higher risk of vector exposure are also considered environmental controls (WHO, 1982; 2013). Environmental management

is focused essentially on reduction of mosquito larval habitats. Therefore, it has strong potential to target several vector populations at the same time, as since mosquito species usually share larval habitats resources, adding effectiveness to methods targeting indoor and outdoor biting vector species (Keiser et al., 2005; Mathews, 2011).

### **1.6. Situation of mosquito-borne diseases in Zanzibar**

Zanzibar has made significant advances in achieving global targets set for mosquito-borne disease control over the last two decades. Malaria prevalence has consistently remained low, ranging from 0% to 0.4% on Pemba and Zanzibar (Unguja) islands, respectively (U.S. President's Malaria Initiative Tanzania (Zanzibar), 2020). The outstanding decline in malaria incidence has been attributed to large-scale implementation of integrated control interventions, consisting of IRS, LLINs, targeted larviciding in urban areas, case management and intermittent prophylactic treatment of infants and pregnant women (Beer et al., 2013; Bjorkman et al., 2019). Currently, Zanzibar archipelago is considered a malaria pre-elimination region (Bjorkman et al., 2019; WHO, 2020b), with residual malaria transmission occurring only in some settings on Zanzibar (Unguja) island (Bjorkman et al., 2019). *Plasmodium falciparum* is the most common malaria parasite, transmission is largely maintained by *An. arabiensis* and *An. funestus* (Haji et al., 2013). The situation of other MBDs and implicated mosquito vector species remains poorly known. However, some studies indicate that lymphatic filariasis (LF), transmitted throughout Africa by *An. arabiensis*, *An. funestus* and *Cx. quinquefasciatus* (White, 1989), rebounding in Zanzibar after more than a decade of zero prevalence (Rebollo et al., 2015). Approximately one million people may be at risk of LF infection in Zanzibar (Rebollo et al., 2015). The burden of mosquito-borne arboviral diseases also remains poorly addressed. However, recent serological surveys have pointed to the fact that DENV and CHIKV may be circulating in local populations (Shauri et al., 2021; Vairo et al., 2012; Vairo et al., 2014). The presence of the vector species *Ae. aegypti* in Zanzibar has also been confirmed, but its role as a vector of any sort of pathogens in the region has yet to be determined. Some studies have suggested that *Aedes*-borne viral disease epidemics may be common throughout rural and urban areas of Zanzibar over the short and long-term (Mweya et al., 2016; Saleh et al., 2020b) (Figure 1.6). Climate and environmental changes and rapid demographic expansion may be potential triggers for mosquito-borne arboviral epidemics, such as dengue (Mweya et al., 2016)

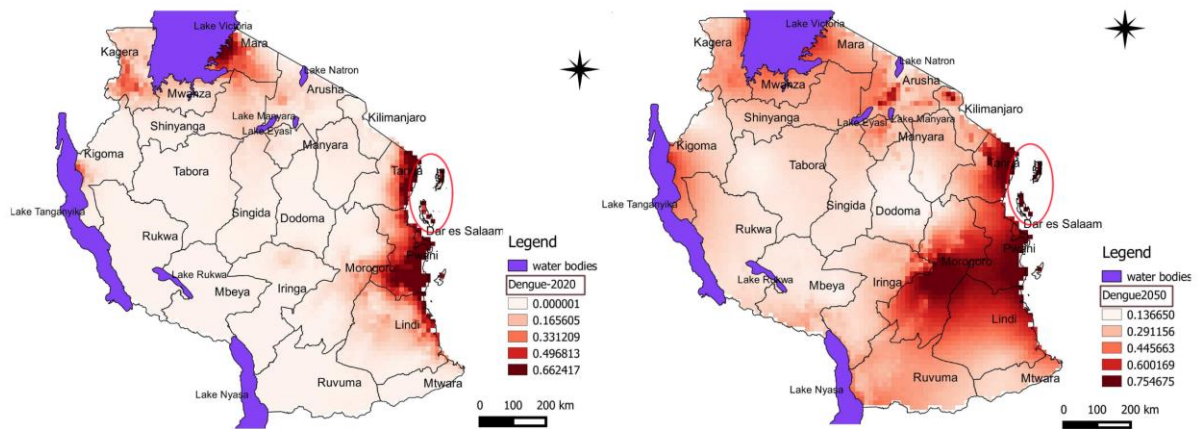


**Figure 1.6.** Spatiotemporal prediction of dengue virus (DENV) outbreaks in Tanzania from 2020 - 2050. Dengue risk projection maps after Mweya et al (2016).

### 1.6. Tourism and mosquito-borne diseases in Zanzibar

Tourism has become one of the most important global sources of revenues. In 2019, travel & tourism contributed to 10.4% of global Gross Domestic Product (GDP) (USD 9.2 trillion) and 10.6% of all jobs (334 million) (WTTC, 2021). Tourism is backbone of development in several insular regions, including in the Zanzibar archipelago (The Revolutionary Government of Zanzibar, 2015). Currently, tourism is the third largest economic sector in Zanzibar, accounting for 27% of GDP, 80% of foreign revenues and 72,000 jobs (The Revolutionary Government of Zanzibar, 2015). However, while tourism produces important economic benefits to the region, this vital sector also generates enormous quantities of municipal solid wastes (MSW) (The Revolutionary Government of Zanzibar, 2015). Nearly 80% of these MSW is poorly managed, particularly in the coastal areas of Zanzibar (The Revolutionary Government of Zanzibar, 2015). These purposeless products, such as plastic containers, tins, buckets, jerry cans, etc., provide suitable environmental conditions for the proliferation of efficient disease vectors and nuisance mosquito species, and consequently persistence of MBDs (Saleh et al., 2018). An eventual MBDs outbreak may compromise several tourism seasons with short and long-run dramatic consequences. For instance, an outbreak of CHIKV in Réunion in 2005 caused nearly €4.5 million loss in tourism revenues in several regions across the archipelago (Deisting & Rey, 2015). Similarly, the 2016 ZIKV epidemics in the South America and the Caribbean caused a loss of international tourism revenue estimated to vary between \$1.8 billion

and \$2.5 billion (United Nation Development Programme, 2017). Therefore, there is an urgent need to implement interventions that will catapult tourism-based development while (at same time) reducing the risk of MBDs epidemics in Zanzibar. As pointed out by Gardner et al (Gardner et al., 2019) taking MBDs and tourism as a coupled system will allow for the assessment of the efficacy of different intervention strategies to inhibit transmission, support the monitoring of the spatiotemporal trajectory of MBD outbreaks, as well as the impact of altered travel patterns in response to mosquito-borne disease risk perceptions or epidemics.

### 1.7. Problem statement

Zanzibar has attained significant gains in terms of MBDs control. Massive scale up of both resources and commitment over the last decades has caused an unprecedented decline in MBDs incidences in the region. These gains have been attributed to scale up of vector control measures and preventive prophylactic treatments of most vulnerable population strata (viz., children and pregnant women) for malaria control (Bjorkman et al., 2019), and also mass drug administration campaigns against lymphatic filariasis control (Rebollo et al., 2015). Success in putting malaria and LF on the verge of elimination, indicates that eradication of MBDs can be achieved soon. Insecticide-based vector control methods are the mainstay of vector control interventions in Zanzibar (Haji et al., 2013). However, the overreliance on chemical control has selected the local vectors into development of resistance, adversely affecting the effectiveness of vector control interventions by mean of IRS and LLINs (Haji et al., 2013; Jones et al., 2012). This has postponed the prospect of MBDs elimination in Zanzibar, despite higher coverage of IRS and LLINs, reinforcing the earlier findings that MBDs transmission cannot be tackled by control measures targeting only a single stage of the mosquito population life cycle, in this case the adult stages. Therefore, integrated application of traditional control tools and approaches that reduce human exposure to mosquito vectors and disease transmission indoors and outdoors are needed. Likewise, effective elimination of MBDs in Zanzibar would require comprehensive implementation of control measures and vector surveillance (Killeen et al., 2002). Currently, hotels in Zanzibar have not been covered by a public mosquito surveillance system and periodic vector control campaigns, albeit reports of mosquito bites at hotel facilities (ZATI, *personal communication*, May 2016). Therefore, we predict that hotel compounds may create refuges for residual vector populations that escape control intervention measures applied in the immediate surrounding communities. This has been supported by an increasing number of complaints on mosquito bites hotels have received from visitors (ZATI,

*personal communication*, May 2016). Moreover, the fear of getting exposed to infectious mosquito bites has been also one of the reasons for negative reviews, and possibly negative publicity, hotels have received. Furthermore, it is one of the reasons for the low interest by tourists to return to the islands (ZATI, *personal communication*, May 2016). Furthermore, fear of getting infected with deadly MBDs such as malaria, dengue and, currently, the infamous ZIKV may also discourage tourists to visit certain regions or force travel companies and tour providers to shun attractive routes with detrimental impact on the budget balance and, consequently, on macroeconomic indicators of the archipelago. On the other hand, hotels also receive visitors from several endemic regions worldwide which may increase the contact between these visitors and local vectors and thus increase the risk of vector exposure to novel MBDs pathogens. The movement of infected host or introduction of susceptible ones may create a suitable landscape to spark MBDs epidemics in Zanzibar. Therefore, an in-depth characterization of residual mosquito population at hotel compounds in Zanzibar and driving factors of vector populations assemblages, dynamics and persistence may help device high precision environmentally sustainable effective mosquito management interventions (Killeen, 2014). Hotels also provide a highly structured and less disturbed microenvironment which may be also ideal for testing alternative insecticide-free mosquito control measures.

## **1.8. Research questions**

This thesis aimed to find the answers for the following research questions:

1. What is the structure, dynamics, and drivers of mosquito community assemblages at hotel compounds in Zanzibar?
2. What is the structure and dynamics of mosquito-larval habitat interaction networks, and the degree of robustness against larval habitat loss at hotel compounds?
3. What are the environmental drivers of dominant mosquito vector species occurrence and abundance at hotel compounds?
4. What are the predominant mosquito control practices at the hotel compounds?
5. What is the status of insecticide susceptibility in the dominant mosquito vectors species at hotel compounds?

## 1.9. Aims

This work aimed at characterizing mosquito community assemblages and factors influencing occurrence and persistence of major mosquito vector populations at hotel compounds in Zanzibar. It also aims at integrating gathered information to develop tools for assessment of the efficacy of appropriate mosquito management interventions at hotel settings in Zanzibar based on produced findings.

## 1.10. Objectives

The main objectives were:

1. To investigate the structure, dynamics, and environmental drivers of mosquito populations assemblages at hotels compounds on the Southeast coastal region of Zanzibar (Unguja) Island.
2. To determine the structure and dynamics of larval habitat utilization networks by mosquito vector populations.
3. To assess the robustness of mosquito communities to designated larval sources elimination strategies.
4. To determine local environment associated factors promoting occurrence and abundance of dominant vector species *Aedes aegypti* and *Ae. bromeliae* at hotel compounds.
5. To outline existing mosquito control practices.
6. To determine the status of insecticide susceptibility and molecular mechanisms underpinning DDT/pyrethroids resistance in *Ae. aegypti* population at hotel compounds.
7. To develop a tool for implementation of hotel-based mosquito larval sources reduction interventions and assessment of mosquito risk exposure at hotel compounds in Zanzibar.

## CHAPTER 2

### **Targeted elimination of species-rich larval habitats can rapidly collapse arbovirus vector mosquito populations at hotel compounds in Zanzibar**

This chapter addresses objectives one to three of the thesis, and it has been published with slight modification as:

Kampango A., Furu P., Sarath D.L., Haji K.A., Konradsen F., Schiøler K.L., Alifrangis M., Weldon C.W. & Saleh F. (2021). Targeted elimination of species-rich larval habitats can rapidly collapse arbovirus vector mosquito populations at hotel compounds in Zanzibar. *Medical and Veterinary Entomology*, **35**, 523-533.

## Abstract

Understanding the dynamics of larval habitat utilization by mosquito communities is crucial for the design of efficient environmental control strategies. We investigated the structure of mosquito communities found at hotel compounds in Zanzibar, networks of mosquito interactions with larval habitats and robustness of mosquito communities against elimination of larval habitats. A total of 23,698 mosquitoes comprising twenty-six species in six genera were found. *Aedes aegypti* (n = 16,207), *Ae. bromeliae*/*Ae. lili* (n = 1,340), *Culex quinquefasciatus* (n = 1,300) and *Eretmapodites quinquevittatus* (n = 659) were the most dominant species.

Ecological network analyses revealed the presence of dominant, larval habitat generalist species (e.g., *Ae. aegypti*), exploiting virtually all types of water holding containers and few larval habitat specialist species (e.g., *Ae. natalensis*, *Orthopodomyia spp*). Analysis of mosquito community robustness to systematic elimination of larval habitats indicate that mosquito populations are highly sensitive to elimination of larval habitats sustaining higher mosquito species diversity.

This study provides insights on potential foci of future mosquito-borne arboviral disease outbreaks in Zanzibar and underscores the need for detailed knowledge on the ecological function of larval habitats for effective mosquito control by larval sources management.

## 2.1. Background

Mosquitoes are the most important vectors of deadly pathogens affecting more than half of the world population (WHO, 2017a). Vector control is the most cost-effective approach for reducing the transmission of mosquito-borne diseases (MBDs) and, for some diseases, is the only available tool to do so (Wilson et al., 2020). Despite significant advances in achieving MBD control targets, residual outdoor malaria transmission persists in endemic regions, particularly in the Zanzibar archipelago (Bjorkman et al., 2019). Concurrently, the emergence and widespread resistance against all approved classes of insecticides in vector populations has persistently postponed the prospect of MBD elimination in the region (Haji et al., 2013; Jones et al., 2012), emphasising the urgent need for novel and adaptable non-chemical environmental vector control practices. Spatiotemporal distribution of mosquito populations and pathogens is greatly affected by variations in climate and environmental factors (Campbell-Lendrum et al., 2015). However, persistence of vector populations over the landscape is largely determined by availability of hosts and larval habitats (Chaves et al., 2010; Franklinos et al., 2019). Therefore, rigorous monitoring of mosquito community assemblages and modulating factors is critical for the design of integrated vector management approaches (Fouet & Kamdem, 2019). Notably, quantitative understanding of the structure and dynamics of mosquito - larval habitat interaction networks are crucial to identify functionally important larval habitats that can be efficiently targeted by environmental control interventions. Moreover, it can be used to predict the magnitude of the impact of larval source management strategies on a mosquito community.

The tourism industry, dominated by hotels, resorts and lodges (henceforth hotels), is the third most important economic sector in Zanzibar, accounting for nearly 27% of gross domestic product, 80% of foreign revenue, and 72,000 jobs (The Revolutionary Government of Zanzibar, 2015). However, hotels in Zanzibar generate large quantities of solid waste (UNICEF, 2018) reported to be associated with higher risk of *Aedes*-borne disease outbreaks in Stone Town, the capital city of Zanzibar (Saleh et al., 2018). The contribution of hotels in creating conditions for the establishment and proliferation of residual vector communities in Tanzania remains unknown. However, the relatively undisturbed environment inside hotel compounds may provide refuges for mosquito taxa of public concern, particularly during unfavourable times when periodic vector control campaigns have been implemented in surrounding communities. This might limit the efficacy of any control measures, as hotels have not been covered by community-wide mosquito control interventions. As circumstantial

evidence for the hypothesis that hotels provide "refuges" for mosquitoes, reports of mosquito bites by visitors have been increasing on these properties. This has been noted as one of the main reasons for negative reviews received by hotels, and a main cause for low interest in returning to the islands (Zanzibar Association of Tourism Investors, *personal communication*, May 2016). Moreover, tourists staying at hotels can import new variants of pathogens from endemic regions around the world. If these new pathogens infect the local mosquito community, it increases the risk of epidemics, with devastating impacts on the tourism-dependent economy of Zanzibar. Therefore, understanding the contribution of hotels to maintenance of residual hotspots of mosquito vector assemblages is crucial for devising effective and ecologically sound control practices.

Our main goals were to investigate the structure, dynamics, and environmental drivers of mosquito populations assemblages at hotels compounds on the Southeast coastal region of Zanzibar Island; determine the structure and dynamics of larval habitat utilization networks by mosquito vector populations and assess the robustness of mosquito communities to designated larval sources elimination strategies. We tested the general hypothesis that there are certain types of larval habitats at studied sites that maintain the stability of mosquito communities. Therefore, targeted elimination of these keystone mosquito sources can dramatically impair mosquito community robustness and cause rapid extinction of mosquito populations. Implications of our findings for the design of non-chemical environmental control (NcEC) interventions in Zanzibar are discussed.

## **2.2. Material and methods**

### **2.2.1. Description of study sites**

This study was carried out at four selected hotels in the South-East coastal region of Zanzibar Island (Figure 2.1). Hotels were selected according to compound size (total residential and non-residential area not less than one hectare), accessibility during lower and higher tourism seasons, willingness to share data and willingness to accept publication of findings. Consent was obtained from hotel management to conduct the study on their properties. For anonymity purposes, hotel names are omitted in this article and, therefore, they are referred to as Hotel A, B, C and D. On this part of the island, the landscape is dominated by moderate forests, open grassland and some wetlands (Mwalusepo et al., 2017). The largest hotel (Hotel B) occupies an area of approximately 28.06 hectares, whilst the remaining hotels encompass an

approximate area of 6.6 hectares (Hotel A), 3.6 hectares (Hotel D), and 2 hectares (Hotel C). The climate of Zanzibar Island is heavily influenced by both southwest monsoon (*Kusi*) and northeast monsoon winds (*Kaskazi*), usually observed from April to October and November to March, respectively. The rainfall regime is divided into two main rainy seasons, the long rainy season (Conteh et al., 2010) usually starts from mid-March to June, whereas the short rainy season (*Vuli*) occurs from November to December. The average monthly precipitation ranges from 30 mm in the winter (July) to 320 mm in the summer (December) and accumulated annual rainfall can reach 1600 mm (Forum, 2019a). Some scattered rain showers can also occur during winter. Daily average temperature oscillates from 22°C in July to 32°C in December (Forum, 2019a). The relative humidity is usually high, with a monthly average ranging from 87% during the long rainy season (March to June) to 76% in November and December (during the short rainy season) and reaching a minimum of 60% during the dry season (January to March and July to October) (Forum, 2019a). The eastern coastal region of Zanzibar Island is relatively arid, making it attractive and ideal for fishing villages, as well as tourist and recreational industries (Forum, 2019b). In general, there is no surface rivers in Zanzibar archipelago. The hydrology of the archipelago comprises chains of underground rivers running through caves. The rivers can eventually re-emerge downstream, in some regions, to feed surface pools of water as natural springs (Hardy et al., 2015).

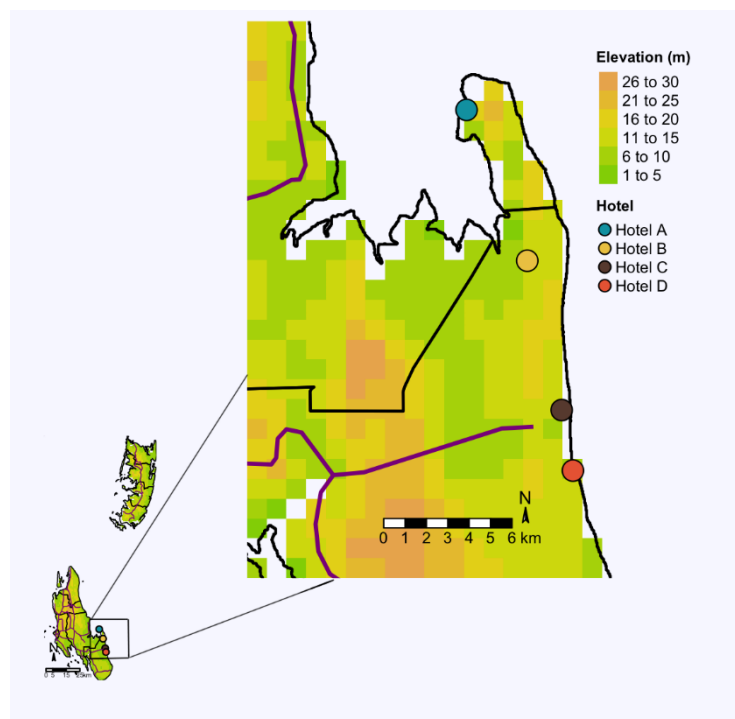


**Figure 2.1.** Map of Zanzibar Island showing the geographical location of the four hotels on the coast of the Southeast region. Zanzibar is the largest and most southern of the two major islands of the Zanzibar archipelago. Dark magenta lines depict main roads. Map made by author, elevations raster and administrative borders shapefile obtained from <https://gadm.org>, roads polygon obtained from <http://www.naturalearthdata.com>.

## 2.2.2. Mosquito sampling strategy

### 2.2.2.1. Sampling of immature mosquito

Mosquito immature stages were monitored for a period of ten-months from September 2018 to October 2019. At each hotel, mosquitoes were surveyed over four to six days each month. The following sampling strategy, adapted from Tun-Lin et al., (1994b) and Manrique-Saide et al., (2011), was adopted according to larval habitat size and water volume. For containers with less than 20 litres of water, all the larvae and pupae were collected by sieving the water directly through a mesh net or collecting them by pipettes. If the water volume was more than 20 litres and emptying the container was not feasible (due to its size or nature), but there was good visibility and relatively low larval and pupal density (less than 100 individuals), then all the specimens were sampled using dippers and sweep nets or bowls. Otherwise, only 10 - 15 dips/sweeps were randomly carried out to collect the specimens. (4) If containers were too deep (e.g., wells) samples were collected using small buckets (approx. 5 litres) suspended by a rope. Ten buckets full of water collected from different sites inside the well were obtained. While in the field, mosquito larvae of well-known predacious species, such as, *Toxorhynchites* species, *Cx. tigripes* and *Eretmapodites* species were separated into different vials to avoid predation on other mosquito larvae. Extra samples of non-predacious mosquito larvae were collected to feed the predacious ones. Containers without mosquito larvae or pupae were considered as potential larval habitats for any local mosquito species only if it had water and physical integrity capable of retaining collected water for at least three consecutive days, which corresponds with the average time required for larvae of several Afrotropical mosquito species to hatch from eggs in the wild (Service, 1990).

### 2.2.2.2. Sampling of adult mosquito

Host-seeking mosquitoes were sampled from 18:00 to 06:00 hours over three days a week, also for a period of ten-months, using CO<sub>2</sub> unbaited CDC light traps (John W. Hock Company, Gainesville, USA). Light traps were hung up inside occupied bedrooms or set up in shaded

places outside the same rooms (WHO, 1975). Resting mosquitoes were sampled by Prokopack aspirators (John W. Hock Company, Gainesville, USA). Resting collections were performed outdoors for 30 minutes, early in the morning (06:00 - 08:00 hours), whereas indoor collections were undertaken inside rooms for 15 minutes. Both outdoor and indoor collections were performed when conditions permitted (e.g., weather conditions, accessibility).

### **2.2.3. Sample processing and identification**

Samples of immature mosquitoes were reared at insectary environmental conditions of temperature ( $27 \pm 2^\circ\text{C}$ ) and relative humidity ( $75 \pm 10\%$ ) until they emerged as adults (WHO, 2016). Larvae were fed ground adult cat food biscuits and kept in a 12:12h light:dark regime until adult emergence. Adult mosquitoes were killed by placing them inside a refrigerator. Mosquitoes were identified morphologically down to species or genus levels using taxonomic keys for Afrotropical mosquito fauna (Coetzee, 2020; Edwards, 1941; Huang, 2004; Jupp, 1986; Service, 1990). Mosquitoes that could not be identified to species morphologically, such as members of species complexes, were placed individually in Eppendorf tubes with silica gel and stored at  $-20^\circ\text{C}$  for molecular typing.

### **2.2.4. Environmental data acquisition**

The environmental variable considered in this study was the normalized difference vegetation index (NDVI), a metric which reflects successional changes of local vegetation cover, and which correlates with fluctuations of mosquito incidence and abundance (Jamison et al., 2015). Daily NDVI records were retrieved from NASA's Terra and Aqua satellites carrying moderate-resolution imaging spectroradiometer sensors (MODIS), via the R package MODISTools (Tuck et al., 2014). Acquired monthly average vegetation data corresponded to the period from September 2018 to October 2019. NDVI data were obtained using the geographical coordinates of each hotel via MODIS product MOD13Q1, which provides global 16-day images at 250-metre spatial resolution. Daily variation of NDVI was sampled in function of the size of studied sites. NDVI ranges from 0 (no vegetation) to 1 (full vegetation growth) (Jamison et al., 2015).

## 2.2.5. Data analysis

### 2.2.5.1. Mosquito community structure

Mosquito community structure was characterized in terms of mosquito abundance, expressed as the total number of specimens collected at each location per month, and mosquito diversity quantified in term of Hill numbers ( $^qD$ ), which convert diversity indices into the effective number of species (ENS) (Chao et al., 2014). We focused on three measures of Hill numbers of order  $q$ , that is,  $q = 0$  (diversity of order 0,  $^0D$ ) quantifies species richness, the total number of observed species in the assemblage,  $q = 1$  is the exponential of Shannon diversity and quantifies the effective number of abundant or equally common species ( $^1D$ ), and  $q = 2$  is the inverse of Gini-Simpson index which quantifies the effective number of highly abundant species ( $^2D$ ) (Chao et al., 2014). The true number of mosquito species (actual richness) occurring at each study site was estimated by Chao1 bias corrected (Chao1 bc) richness estimator using the R package, iNext (Hsieh et al., 2016). Exploratory analysis indicated that the composition of adult mosquito catches closely approximated nested subsets of larval collection samples (see appendix Table A2.1 and Table A2.2). Additionally, some mosquito species were exclusively detected by one type of collection method and *vice versa*. As such, we pooled both adult and immature mosquito catches to overcome the problem of differential detectability between sampling methods, achieve more representative samples of mosquito species and, ultimately, obtain relatively accurate descriptions of mosquito species composition, estimation of the true mosquito richness and other already diversity indices. A similar approach has been applied with other insect communities (Colwell & Coddington, 1994). No further inferential analysis was performed on adult mosquito catches due to a very small number of individuals caught. Therefore, subsequent analyses were concentrated only on immature mosquito samples. The completeness and coverage of our sampling design was verified by species-accumulation curves (Chao et al., 2014).

### 2.2.5.2. Spatiotemporal variation in mosquito composition

Multivariate abundance generalized linear models (manyGLM) (Wang et al., 2012) were applied on immature mosquito catches to determine the magnitude of variations in mosquito composition between hotels, season and relationship with local variation in vegetation cover. Multivariate abundance models were fitted using the package mvabund (Wang et al., 2012). The manyGLM fits a global multivariate model and several separate univariate GLMs

according to the number of taxa in the community matrix. Inference is made by summation of all univariate model likelihood ratio (LR) statistics (Wang et al., 2012). Therefore, the percentage each taxon contributes to global community response can be derived by comparisons between the LR from the deviance of the univariate model and the global deviance (*sum-of-LR*) (Wang et al., 2012). A cubic spline smoothing function was applied to capture the possible nonlinear effect NDVI on mosquito counts. Mosquito counts were assumed to follow a negative binomial (NB) distribution with log-link function to account for possible over-dispersion. Model compliance to distributional assumptions was assessed by visual investigation of scatter plots of observed residuals against the fitted residuals (Wang et al., 2012).

### 2.2.5.3. Mosquito - larval habitat interaction networks

Ecological interaction network analyses (EIN) were applied to determine the structure and dynamics of mosquito - larval habitat interaction networks, and to investigate the response of mosquito populations to induced elimination of larval habitats resulting from environmental control interventions. The followings standard network metrics were calculated: (i) network-level specialization index ( $H_2^*$ ); (ii) species-level specialization index ( $d'$ ) (Blüthgen et al., 2006); (iii) species degrees index (iv); network strength (Bascompte et al., 2006) (v) and network robustness ( $R$ ) (Memmott et al., 2004). For further details on interpretation of network metrics see Table 2.1.

Envisaging future mosquito control interventions by larval source management at studied sites, we simulated three potentially realistic tactics of systematic extinction of larval habitats and evaluated the probable impact on mosquito community. The first tactic we examined (denoted here as R0) was random removal of larval habitats irrespective of frequency or number of links (i.e., total number of mosquito species that frequently exploit the resources as larval sites). This strategy was considered as the 'null model' with which to contrast other possible types of systematic elimination (Memmott et al., 2004). The second tactic (R1) involved targeted extinction of larval habitats according to their prevalence in the environment, starting from the most prevalent to the least prevalent larval habitats. For the third strategy (R2), we simulated targeted removal of larval sites based on the number of species depending on them to breed (i.e., number of links), starting from the most connected to the least connected ones. Network robustness ( $R$ ) was calculated as the area below the attack tolerance curve (ATC) representing the proportion of mosquito species persisting as a function of eliminated larval habitats (Burgos

et al., 2007; Memmott et al., 2004). The significance of observed network metrics was determined by comparison against expectations generated by null model algorithms proposed by Patefield (1981) and Vázquez et al., (2007). As such, 1000 random network matrices were generated using the null model algorithms. To allow direct comparisons, observed network metrics were converted into z-scores determined as  $(\text{Observed}_{\text{metric}} - \text{Null}_{\text{metric}} \text{ mean}) / \text{Null}_{\text{metric}} \text{ standard deviation}$ . Z-score values higher than or equal to 2 denote observed metrics significantly higher than expected by chance (Dormann et al., 2009). Ecological interaction network metrics were estimated using the package bipartite (Dormann et al., 2009). All the data processing tasks, and statistical analyses were implemented with R software version 4.0.2 (R Core Team, 2022).

**Table 2.1.** Ecological network topology metrics used to describe the structure and dynamics of mosquito community and larval site interaction networks.

| Network metric                          | Definition/Interpretation                                                                                                                                                                                                                      | Reference               |
|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| Network-level specialisation ( $H_2'$ ) | Applied to quantify the overall degree of mosquito community specialization in utilization of larval habitats. $H_2' = 0$ suggests generalist community and $H_2' = 1$ indicates specialist (selective) community.                             | Blüthgen et al. (2006)  |
| Species-level specialization ( $d'$ )   | Applied to quantify the degree of mosquito species specialisation in use of larval habitat relative to other species in the network. $d' = 0$ indicates unselective/generalist population and $d' = 1$ indicates selective/specialist species. | Blüthgen et al. (2006)  |
| Species degrees                         | Indicates the number of interactions with other species in the network. Applied here to determine the number of mosquito species interactions with larval habitats                                                                             | Bascompte et al. (2006) |
| Species strength                        | Quantifies the importance of a resource for the entire network. Here it was applied to quantify the importance of a larva habitat to mosquito community stability.                                                                             | Bascompte et al. (2006) |

|                    |                                                                                                                                                                                                                                    |                       |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Robustness ( $R$ ) | Applied to determine the tolerance of mosquito community to elimination of larval habitats. $R = 0$ indicates higher susceptibility to loss of larval habitats and $R = 1$ indicates higher resistance to loss of larval habitats. | Memmott et al. (2004) |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|

## 2.3. Results

### 2.3.1. Mosquito community structure and dynamics

In total 23,698 mosquitoes were collected from both immature and adult mosquito surveys. Larval collections yielded the highest number of specimens, accounting for nearly 94.1% ( $n = 22,307$ ) of all specimens sampled, followed by samples using the Prokopack aspirator and CDC light trap, which contributed 4.69% ( $n = 1,112$ ) and 1.14% ( $n = 269$ ), respectively. Twenty-six mosquito species were identified from pooled immature and adult mosquito catches (Table A2.1 and Table A2.2). Both larval and adult mosquito samples contained important vector species (appendix Table A2.3). *Aedes aegypti* was by far the most abundant species collected as larvae from all four hotels and, together with *Ae. bromeliae*/*Ae. lili*, *Ae. metallicus* and *Cx. quinquefasciatus*, represented 88.77% ( $n = 19,801$ ) of all mosquitoes collected (Table A2.3). *Culex quinquefasciatus* was the most common species among the adult catches, accounting for 88% ( $n = 1,224$ ) of all specimens (Table A2.2). Thirteen mosquito species were detected at Hotel C, 14 species (Hotel B) and 17 species (Hotel A and Hotel D) from pooled immature and adult samples (Table 2.2). At all four hotels the observed number of species was within the range of the estimated richness ( $^0D$ ) predicted using the Chao unbiased richness estimator. The highest mosquito diversity was observed at Hotel D, followed by Hotel A, D and C (Table 2.2).

**Table 2.2.** Total abundance and diversity (expressed as Hills numbers) of mosquito catches from study sites as revealed by combined immature and adult catches.

| Hotel   | Total sampled | Observed richness | Estimated richness | Shannon diversity | Simpson diversity |
|---------|---------------|-------------------|--------------------|-------------------|-------------------|
| Hotel A | 6533          | 17                | 17 (17.0 – 18.0)   | 3.1 (3.1 - 3.2)   | 1.75 (1.75-1.81)  |
| Hotel B | 3974          | 14                | 14.5 (14.0 - 22.4) | 2.1 (2.1 - 2.2)   | 1.53 (1.53-1.56)  |

|         |      |    |                    |                 |                  |
|---------|------|----|--------------------|-----------------|------------------|
| Hotel C | 3497 | 13 | 13 (13.0 - 14.6)   | 2.1 (2.1 - 2.2) | 1.49 (1.49-1.54) |
| Hotel D | 8303 | 17 | 17.5 (17.0 - 25.4) | 3.6 (3.6 - 3.7) | 2.29 (2.29-2.34) |

The sample-size-based rarefaction curves fitted on each hotel mosquito community reached an asymptote after a minimum sample size of 2,000 individuals, indicating that our sampling design was able to detect all possible mosquito taxa occurring at each studied site (Figure 2.2).

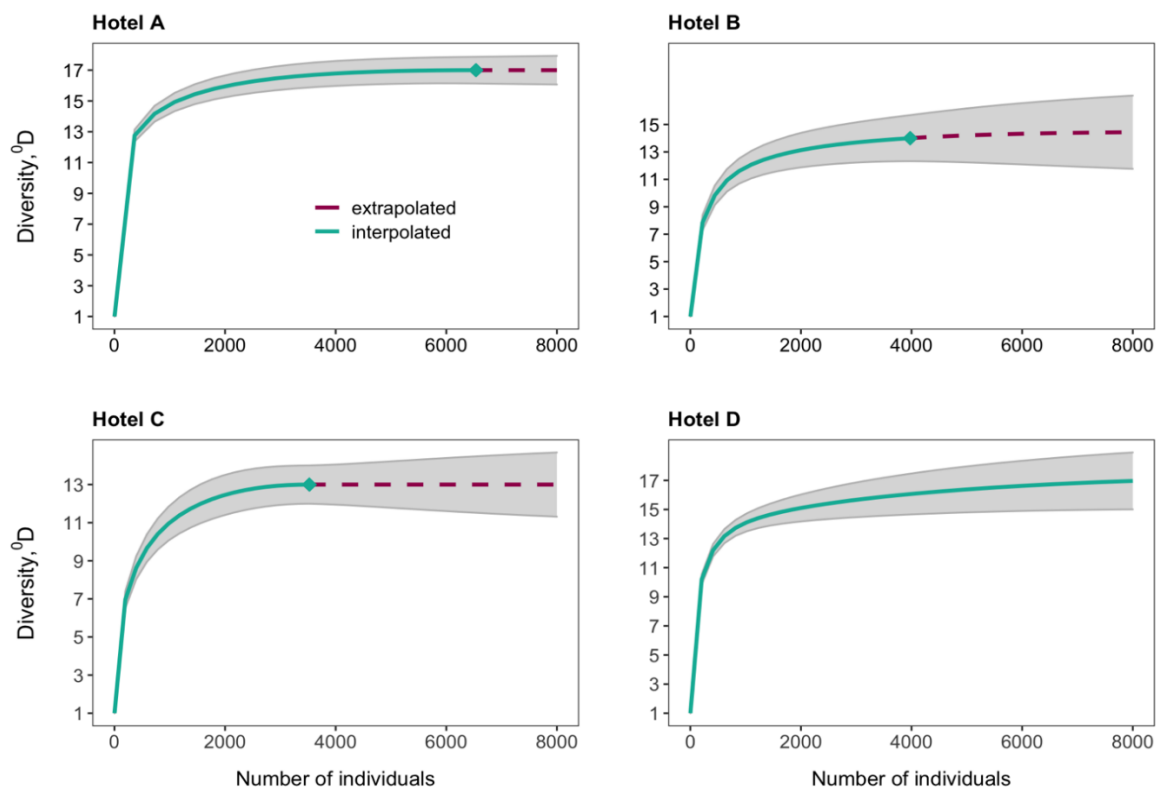


**Figure 2.2.** Sample-size-based rarefaction (solid lines) and extrapolation curve (dashed lines) of mosquito diversity based on the Hill index ( $q = 0$ ). The shaded grey area represents the 95% confidence intervals obtained by the bootstrap method based on 200 replicates.

Results also indicate that mosquito abundance peaks during the long rainy season (around May and June), whereas mosquito species diversity ( $^0D$ ,  $^1D$  and  $^2D$ ) peaks around one to three months later after the end of the long rainy season (July - August) (Figure 2.3). Consistent with this, there was a strong one-month delayed dependence of richness on mosquito abundance (Spearman correlation:  $r = 0.61$ ,  $p < 0.001$ )

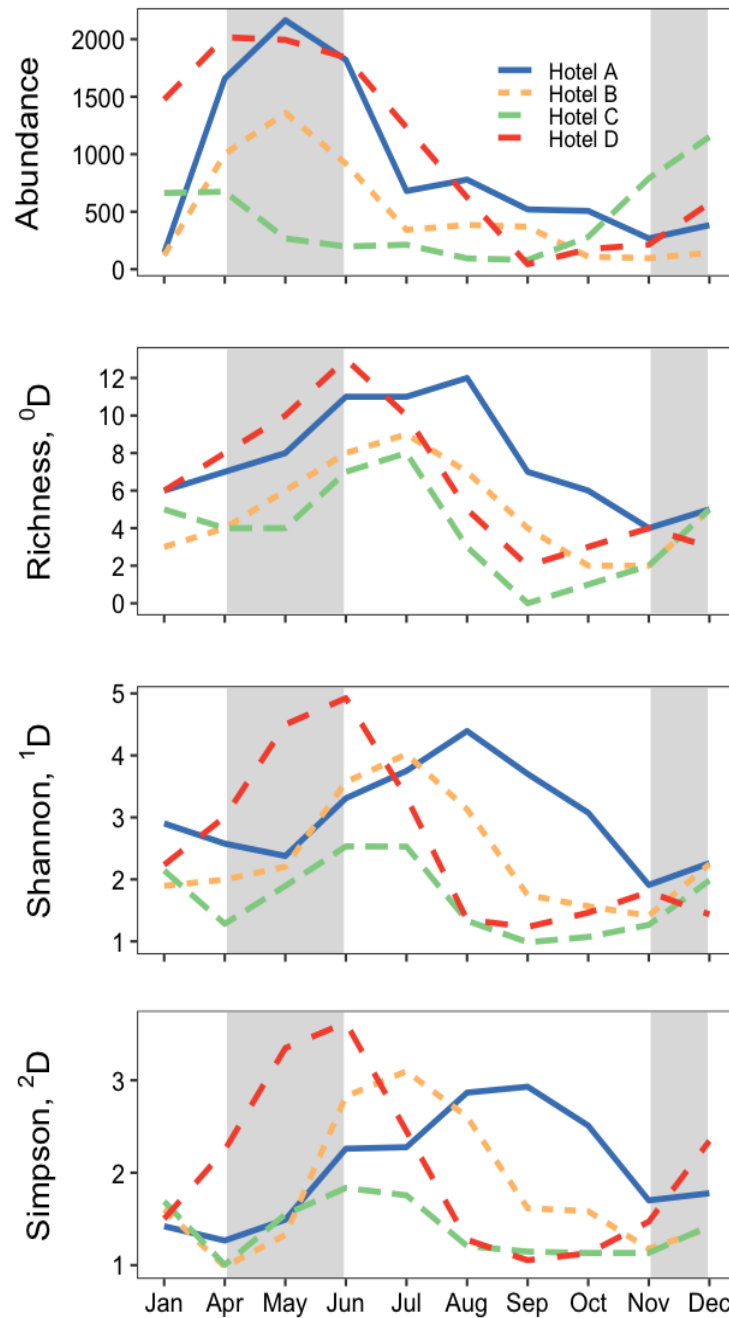


**Figure 2.3.** Temporal dynamics of mosquito community abundance and diversity expressed as net mosquito richness ( $^0D$ ), Shannon ( $^1D$ ) and Simpson ( $^2D$ ) diversities, expressed as the effective number of species (Hill's number). Shaded rectangles indicate the long (April – Jun) and short (November – December) rainy season periods.

The multivariate abundance GLM analysis (manyGLM) showed significant variability in mosquito composition (species turnover) between hotels and season, and a significant nonlinear effect of NDVI (Table 2.3, Figure 2.3). However, the magnitude of covariate effects on mosquito assemblages was species-specific (Table 2.3). Additionally, mosquito abundance and richness also increased nonlinearly with increasing of vegetation cover (NDVI index). However, NDVI values above 0.55 did not significantly explain variability in abundance and richness (Figure 2.4).

**Table 2.3.** Summary of the effects of hotel, season, and vegetation cover (NDVI) on mosquito assemblage abundance and species composition from larval collections.

| Variable     | Residual<br>df | df | Deviance | <i>p-values</i> | Species with significant<br>contribution to total variability                                                                                                                                                                  |
|--------------|----------------|----|----------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hotel        | 27             | 3  | 208.69   | 0.001           | <i>Ae. aegypti</i> (16.51%), <i>Ae. simpsoni</i> (14.02%), <i>Ae. metallicus</i> (23.07%), <i>Ae. bromeliae</i> (17.55%), <i>Er. quinquevittatus</i> (17.90%), <i>Er. subsimplicipes</i> (13.61%), <i>Cx. tigripes</i> (8.39%) |
| Season       | 26             | 1  | 40.61    | 0.04            | <i>Ae. bromeliae/Ae. lillii</i> (5.36%); <i>Er. chrysogaster</i> (4.78%),                                                                                                                                                      |
| Hotel:Season | 23             | 3  | 73.80    | 0.018           | <i>Er. subsimplicipes</i> (6.39%), <i>Cx. quinquefasciatus</i> (22.70%)                                                                                                                                                        |
| bs(NDVI)     | 24             | 4  | 195.34   | 0.001           | <i>Ae. aegypti</i> (9.58%), <i>Ae. bromeliae/Ae. lillii</i> (14.66%), <i>Er. subsimplicipes</i> (13.01%), <i>Er. conchobius</i> (16.93%)                                                                                       |

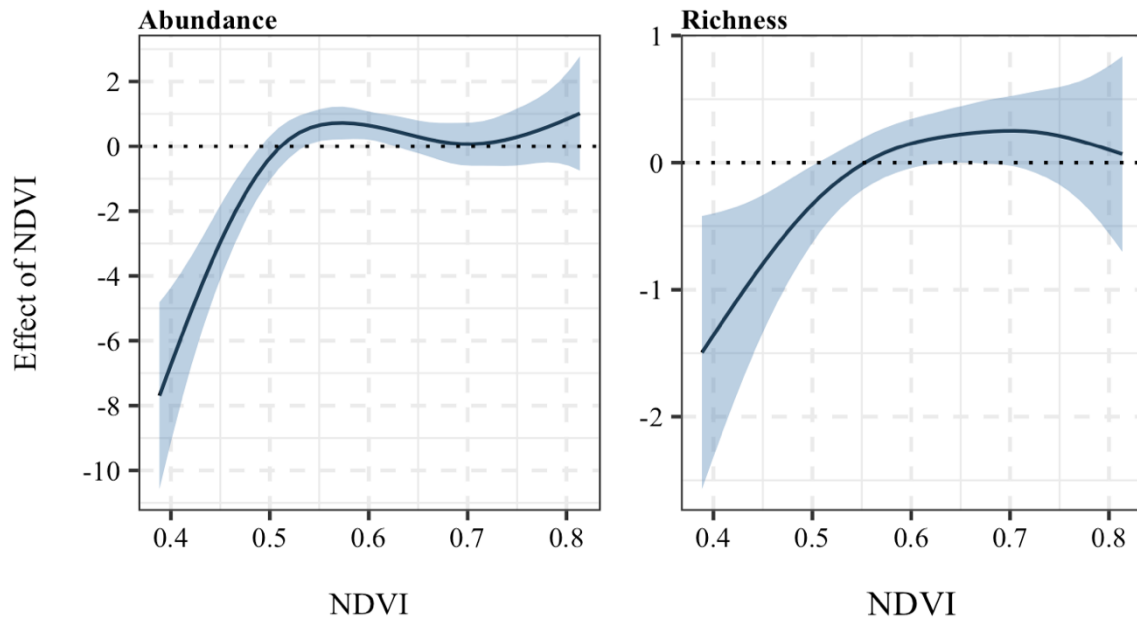


**Figure 2.4.** Effect of vegetation cover variation on mosquito abundance and richness. NDVI index values below 0.5 were significantly associated with lower abundance and species richness. NDVI values above 0.55 added no significant variability.

### 2.3.2. Structure and dynamics of mosquito – larval habitat interaction networks

The topology and seasonal dynamics of mosquito – larval habitat interaction networks are shown in Figure 2.5. The rainy season interaction network comprised eighteen mosquito species and eleven types/groups of larval habitats (Figure 2.5), whereas in the dry season the network comprised nineteen mosquito species sharing 12 types of larval habitats (Figure 2.4). Interaction networks were dominated by abundant larval habitat generalist species, which can exploit virtually all types of larval habitats, and less abundant larval habitat specialist species (Figure 2.5). There was indication of season-driven specialization degree in larval habitat use both at species-level (Table 2.4 and Table 2.5) and network levels. Observed network-level specialization indices were  $H_2' = 0.115$  ( $p < 0.001$ ) and  $H_2' = 0.276$  ( $p < 0.001$ ) in the rainy season and dry season, respectively. *Aedes aegypti* was the most prominent larval habitat generalist irrespective of the season. This vector species was found associated with all eleven types/groups of larval habitats identified in the rainy season, and in 83% of all habitats identified in the dry season.

**Table 2.4.** Focal specialization index ( $d'$ ) of mosquito populations observed during rainy season. Species showing  $d'$  significantly higher than null model expectations are highlighted in grey.

| Species                              | Observed $d'$ | Null model I (Patefield)    |          | Null model II (Vaznull)     |         |
|--------------------------------------|---------------|-----------------------------|----------|-----------------------------|---------|
|                                      |               | Expected $d'$ ( $\pm$ 95CI) | z-scores | Expected $d'$ ( $\pm$ 95CI) | z-score |
| <i>Aedes aegypti</i>                 | 0.05          | 0.021 (0-0.144)             | 0.804    | 0.042 (0-0.295)             | 0.116   |
| <i>Aedes simpsoni</i>                | 0.09          | 0.017 (0-0.128)             | 1.821    | 0.036 (0-0.273)             | 0.618   |
| <i>Aedes metallicus</i>              | 0.03          | 0.023 (0-0.159)             | 0.374    | 0.045 (0-0.363)             | -0.096  |
| <i>Aedes vittatus</i>                | 0.05          | 0.021 (0-0.131)             | 0.952    | 0.04 (0-0.28)               | 0.189   |
| <i>Aedes unilineatus</i>             | 0.00          | 0.016 (0-0.069)             | -0.511   | 0.036 (0-0.275)             | -0.534  |
| <i>Aedes bromeliae/lilii</i>         | 0.07          | 0.017 (0-0.13)              | 1.486    | 0.033 (0-0.271)             | 0.453   |
| <i>Aedes calceatus</i>               | 0.08          | 0.022 (0-0.144)             | 1.796    | 0.045 (0-0.319)             | 0.606   |
| <i>Eretmapodites quinquevittatus</i> | 0.12          | 0.018 (0-0.116)             | 2.806    | 0.035 (0-0.268)             | 1.106   |
| <i>Eretmapodites subsimplicipes</i>  | 0.05          | 0.02 (0-0.132)              | 0.882    | 0.046 (0-0.308)             | 0.155   |
| <i>Eretmapodites conchobius</i>      | 0.13          | 0.021 (0-0.131)             | 2.972    | 0.043 (0-0.365)             | 1.187   |
| <i>Eretmapodites inornatus</i>       | 0.01          | 0.017 (0-0.097)             | -0.232   | 0.036 (0-0.277)             | -0.396  |
| <i>Culex quinquefasciatus</i>        | 0.27          | 0.016 (0-0.093)             | 6.707    | 0.035 (0-0.278)             | 3.033   |
| <i>Culex nebulosus</i>               | 0.00          | 0.016 (0-0.069)             | -0.511   | 0.04 (0-0.299)              | -0.534  |
| <i>Culex tigripes</i>                | 0.09          | 0.016 (0-0.108)             | 1.914    | 0.035 (0-0.273)             | 0.664   |
| <i>Culex cinereus</i>                | 0.33          | 0.021 (0-0.141)             | 8.433    | 0.041 (0-0.283)             | 3.886   |
| <i>Anopheles gambiae</i>             | 0.07          | 0.021 (0-0.134)             | 1.402    | 0.043 (0-0.3)               | 0.412   |
| <i>Toxorhynchites brevipalpis</i>    | 0.05          | 0.017 (0-0.116)             | 0.970    | 0.039 (0-0.286)             | 0.198   |
| <i>Orthopodomyia spp</i>             | 0.56          | 0.02 (0-0.143)              | 14.737   | 0.046 (0-0.364)             | 7.002   |

**Table 2.5.** Focal specialization index ( $d'$ ) of mosquito populations observed during the dry season. Species showing  $d'$  significantly higher than expected by null models are highlighted in grey.

| Species                              | Observed $d'$ | Null model I (Patefield)    |         | Null model II (Vazques)     |         |
|--------------------------------------|---------------|-----------------------------|---------|-----------------------------|---------|
|                                      |               | Expected $d'$ ( $\pm$ 95CI) | z-score | Expected $d'$ ( $\pm$ 95CI) | z-score |
| <i>Aedes aegypti</i>                 | 0.121         | 0.02 (0-0.098)              | 3.878   | 0.058 (0-0.425)             | 0.585   |
| <i>Aedes simpsoni</i>                | 0.047         | 0.018 (0-0.074)             | 1.055   | 0.062 (0-0.423)             | -0.089  |
| <i>Aedes metallicus</i>              | 0.002         | 0.019 (0-0.091)             | -0.657  | 0.059 (0-0.405)             | -0.498  |
| <i>Aedes africanus</i>               | 0.005         | 0.018 (0-0.073)             | -0.560  | 0.055 (0-0.41)              | -0.475  |
| <i>Aedes bromeliae/lilii</i>         | 0.101         | 0.02 (0-0.097)              | 3.127   | 0.058 (0-0.427)             | 0.406   |
| <i>Aedes calceatus</i>               | 0.129         | 0.018 (0-0.08)              | 4.193   | 0.056 (0-0.394)             | 0.660   |
| <i>Aedes heischi</i>                 | 0.082         | 0.018 (0-0.091)             | 2.379   | 0.064 (0-0.413)             | 0.227   |
| <i>Aedes natalensis</i>              | 0.308         | 0.019 (0-0.089)             | 10.994  | 0.054 (0-0.393)             | 2.284   |
| <i>Eretmapodites quinquevittatus</i> | 0.079         | 0.02 (0-0.105)              | 2.265   | 0.057 (0-0.405)             | 0.200   |
| <i>Eretmapodites subsimplicipes</i>  | 0.101         | 0.019 (0-0.092)             | 3.093   | 0.051 (0-0.399)             | 0.398   |
| <i>Eretmapodites inornatus</i>       | 0.052         | 0.02 (0-0.095)              | 1.244   | 0.052 (0-0.389)             | -0.044  |
| <i>Eretmapodites chrysogaster</i>    | 0.066         | 0.019 (0-0.084)             | 1.768   | 0.054 (0-0.394)             | 0.081   |
| <i>Culex quinquefasciatus</i>        | 0.485         | 0.019 (0-0.078)             | 17.705  | 0.057 (0-0.413)             | 3.886   |
| <i>Culex pipiens</i>                 | 0.421         | 0.02 (0-0.094)              | 15.283  | 0.061 (0-0.442)             | 3.307   |
| <i>Culex tigripes</i>                | 0.039         | 0.02 (0-0.093)              | 0.750   | 0.058 (0-0.421)             | -0.162  |
| <i>Culex cinerellus</i>              | 0.456         | 0.019 (0-0.088)             | 16.631  | 0.054 (0-0.409)             | 3.629   |
| <i>Culex cinereus</i>                | 0.434         | 0.02 (0-0.08)               | 15.761  | 0.056 (0-0.411)             | 3.421   |
| <i>Toxorhynchites brevipalpis</i>    | 0.216         | 0.02 (0-0.1)                | 7.502   | 0.057 (0-0.4)               | 1.450   |
| <i>Orthopodomyia spp</i>             | 0.614         | 0.02 (0-0.095)              | 22.620  | 0.057 (0-0.418)             | 5.059   |

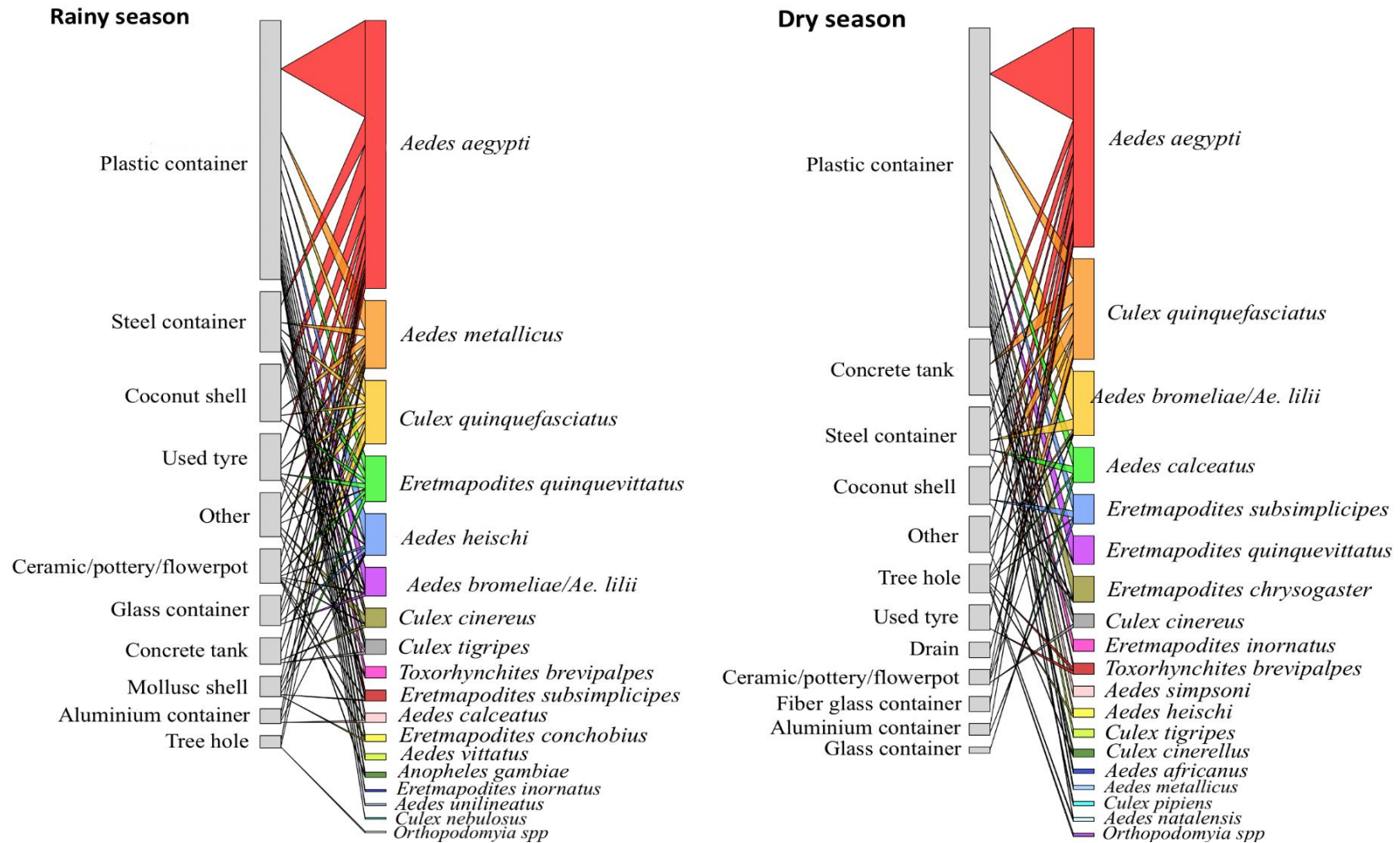


**Figure 2.5.** Quantitative bipartite interaction networks involving mosquito populations and larval habitats identified at studied hotel compounds during rainy and dry seasons. Each rectangle size represents proportional abundance of larval habitats (left) and mosquito species (right). The triangles represent interaction and triangle size is proportional to the frequency of interactions. The rectangles of species nodes and interactions triangles were assigned the same colour to highlight the number of links of each species (higher node) to larval habitats (lower nodes).

### 2.3.3. Mosquito community robustness to larval habitat elimination

Network analyses indicated that plastic containers were the most important larval habitat resource on which mosquito communities depended for breeding (Table 2.6). Other resources such as three holes and concrete tanks were also significant for mosquito community stability, in the dry season (Table 2.6).

**Table 2.6.** Relative dependence of mosquito population to larval sites identified at four hotels studied Zanzibar Island (see Material and Methods).

| Habitat                   | Strength     |            |
|---------------------------|--------------|------------|
|                           | Rainy season | Dry season |
| Plastic container         | 13.38        | 11.80      |
| Used tyre                 | 0.21         | 0.05       |
| Steel container           | 0.57         | 0.25       |
| Fibre glass container     |              | 0.02       |
| Cement tank               | 0.46         | 2.96       |
| Tree hole                 | 1.01         | 1.88       |
| Coconut shell             | 0.26         | 0.41       |
| Aluminium container       | 0.13         | 0.07       |
| Ceramic/pottery/flowerpot | 0.41         | 0.19       |
| Glass container           | 0.12         | 0.08       |
| Drain                     |              | 0.13       |
| Mollusc shell             | 0.31         |            |
| Other                     | 1.14         | 0.15       |

Simulations of network robustness to induced elimination of larval habitats indicated that the mosquito communities were less tolerant to the elimination of highly connected larval habitats (e.g., plastic containers). Eradication of such types of resources sustaining higher mosquito diversity in the community would cause significant disruption of mosquito community structure leading to more rapid extinction of mosquito species at studied sites than removing larval habitats randomly or prioritising only the most abundant ones (Figure 2.6). The highest impact of larval habitat elimination was predicted in the dry season (Figure 2.6).

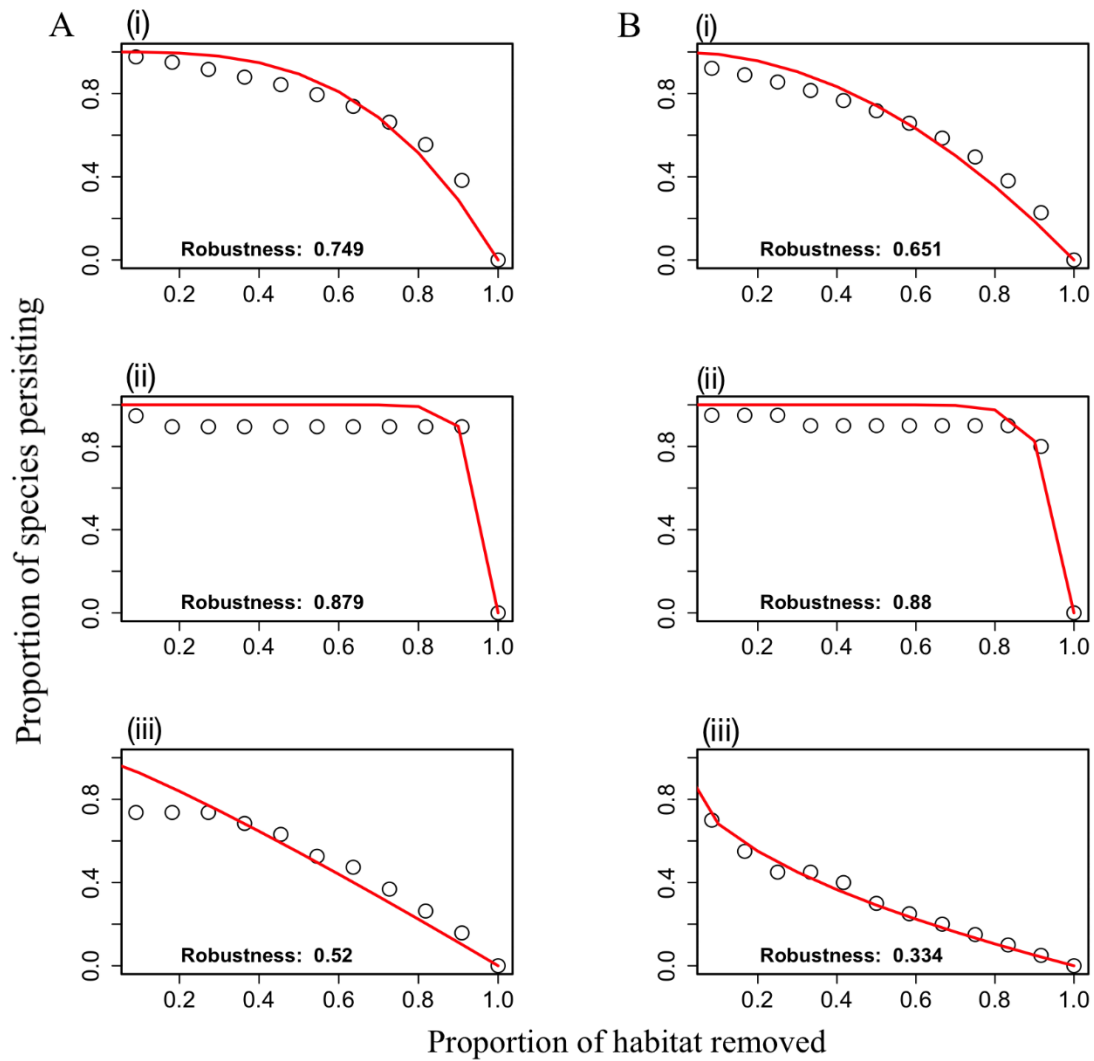


**Figure 2.6.** Mosquito community robustness to targeted elimination of larval habitat during rainy season (A) and dry season (B) considering three strategies of larval habitats removal. The removal strategies are: (i) random removal, ignoring larval habitat abundance and strength (species richness *per capita*) in the network; (ii) removal based on larval habitat abundance, and (iii) removal based on larval habitat strength, starting from the most to least connected larval habitat. Loss of higher linked (keystone) larval habitats tend to cause rapid collapse of the network when compared to random extinction and extinction strategy prioritizing most abundant larval sites.

### 3.4. Discussion

Entomological surveillance and mosquito control interventions have traditionally targeted households and peri-domestic environments (WHO, 2017a), with limited focus on properties managed by the private or non-government sectors, such as hotels, resorts, lodges and others. This has perpetuated the gap in our knowledge regarding the potential of these micromanaged non-public infrastructures to initiate MBD outbreaks of public health concern. We found that hotels in Zanzibar sustain diverse assemblages of both vector and nuisance mosquito species. This is important because consistent surveillance and characterisation of vector species dynamics and drivers of mosquito population persistence in the environment is critical for tailoring precise and context specific environmental control measures that can effectively target all fractions of known vector populations and nuisance species. Persistence of nuisance mosquitoes can potentially undermine public perception of control approaches, even if the most important vector species have been successfully eradicated (Killeen et al., 2007). Our data also show consistent time lags of one to three months between the peak of mosquito abundance and mosquito diversity at each study site. The late peak in diversity (July - August) suggests that mosquito communities reach stability shortly after the end of the rainy season, probably favoured by reduced frequency of natural disturbance events (e.g., rainstorms), which can cause dramatic losses of mosquito immature stages (Paaajmans et al., 2007). This hypothesis may hold in locations such as Zanzibar, where there are no surface rivers or large collections of permanent water bodies (Hardy et al., 2015). Therefore, rainfall can either drive or limit the propagation of local mosquito populations, depending on the amount and frequency, as observed elsewhere (Shaman & Day, 2007). Our findings also suggest that the risk of MBD exposure can be consistently higher even one to three months after the end of the rainy season. Quantitative understanding of the local dynamics of mosquito community composition can provide valuable information on environmental risks of emergent pathogen exposure, and the potential risks associated with the introduction of invasive vector species. We observed significant site-to-site and seasonal variability in mosquito composition, which may reflect differential response to local variations of microclimate and environmental characteristics. We also observed a significant nonlinear effect of vegetation cover (reflected by variation of NDVI index) on mosquito community abundance and diversity. The NDVI index is an indicator of local vegetation productivity and spatiotemporal dynamics, with high values indicating higher vegetation cover (Pettorelli et al., 2005). Vegetation cover may indirectly increase mosquito

abundance and diversity by creating suitable conditions for establishment and survival of mosquito populations. Increasing vegetation cover may, for instance, reduce evaporation rate of standing water from rainfall, gardening, or house-refuse and, thereby, contribute towards availability and persistence of potentially suitable larval habitats (Sauer et al., 2021). Vegetation can also create suitable mosquito resting habitats and increase availability of key energetic resources such as nectar (Peach & Gries, 2020). Several studies have also reported a significant positive association between NDVI and mosquito presence, abundance and diversity (Ferraguti et al., 2016; Juri et al., 2015; Roiz et al., 2015), and MBD prevalence (Jamison et al., 2015).

Ecological network analyses identified small groups of abundant larval habitat generalist vector species, such as *Ae. aegypti*, *Ae. bromeliae*/*Ae. lillii*, *Ae. simpsoni*, *Ae. metallicus*, and *Er. quinquevittatus*, that can colonize virtually all types of available water holding artefacts as larval habitats. These observations concur with what is known about oviposition habits of some of these mosquito species, such as, e.g., *Ae. aegypti* group (Reiter, 2007) and *Er. quinquevittatus* (Lounibos, 1980). It has been argued that this opportunistic oviposition behaviour may be an evolutionary adaptation to maximize the survivorship and fitness of immature stages. It also facilitates propagation over the landscape in the presence of highly ephemeral larval habitats (Reiter, 2007). In contrast, there is also a group of larval habitat specialist mosquito species, such as, *Ae. natalensis* and *Ortopodomyia spp*, which in the present study were found strictly exploiting tree holes in co-occurrence with some generalist species. This type of ecological network topology has been observed in other insect taxa and may be a co-evolutionary adaptation for mosquito communities to resist extreme events, such as habitat disturbances (Bascompte et al., 2006; Vázquez & Aizen, 2004). The ability of generalist species to successfully colonize virtually all types of larval habitats implies that control of this species guild may require much more effort than control of specialist species. However, our results also show an increasing tendency for specialization in larval habitat use during the dry season compared to the rainy season, implying that larval habitat generalist populations, which are the most abundant, may be more vulnerable to control measures targeting mosquito sources in the dry season. What drives phenological specialization in resource use remains poorly understood. However, we can tentatively hypothesize that factors such as competition for disproportionately available larval habitats and behavioural avoidance of predation might be potential drivers in our cases. The size, frequency and number of larval habitats are expected to decrease in the dry season, so that some mosquito-larval habitat ecological interactions may

be lost due to natural extinction of larval habitats. Therefore, shortage of larval resources may increase the rate of inter-specific competition forcing some mosquito species to avoid oviposition in larval habitats with competitors or predator species (Kesavaraju & Juliano, 2004; Schneider et al., 2000).

The impact of environmental control interventions by mosquito larval sources have been traditionally evaluated for a restricted group of well-known vector species. Therefore, mosquito community-wide effects of such types of interventions remain poorly understood. Our simulations of mosquito community tolerance to induced elimination of larval habitats indicate that mosquito populations are most vulnerable to elimination of highly connected (i.e., species-rich) larval habitats (e.g., plastic containers, ceramic pots, coconut shells and tree holes). This is particularly the case in the dry season. Eradication of such sources of higher mosquito diversity may cause rapid extinction of mosquito populations at studied sites. This finding implies that mosquito control interventions by larval source management should prioritise targeted elimination of ecologically functional larval habitats, not just based on larval habitat prevalence. Our results also indicate that the impact of removing ecologically functional larval habitats will be higher in both the rainy and dry seasons compared to other simulated control strategies. However, it is worth mentioning that ecological interaction networks are dynamic systems, so the possibility of adaptive rewiring of lost interactions that have been broken by control measures cannot be ruled out, as observed in other insect taxa (CaraDonna et al., 2017). Therefore, experimental trials to investigate the response of mosquito communities to induced disturbance by elimination of functionally important larval habitats is encouraged before area-wide implementation of larval source management interventions and continued surveillance efforts.

## **2.5. Concluding remarks**

Our findings show that hotels in Zanzibar can sustain a high diversity of vectors and nuisance mosquito species showing predictable interactions with identified larval habitats. Therefore, coordinated mosquito control strategies between tourism and public health sectors on the island are encouraged. Mosquito communities are highly vulnerable to elimination of larval habitats sustaining higher mosquito species richness, making larval source management a potential candidate for future environmental control interventions. Our findings also highlight the need for further investigation on the ecological function of larval habitats for effective mosquito

management by environmental control approaches. This study has focused on mosquito communities within hotel compounds, but future studies in other settings are encouraged.

## **2.6. Acknowledgements**

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## CHAPTER 3

### **Risk factors for occurrence and abundance of *Aedes aegypti* and *Aedes bromeliae* at hotel compounds in Zanzibar**

This chapter addresses objective four of the thesis, and it has been published with slight modification as:

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## Abstract

A field survey was performed to investigate local environmental factors promoting occurrence and abundance of *Aedes aegypti* and *Ae. bromeliae* mosquitoes at hotel compounds in the south-east coastal region of Zanzibar Island. The potential risk factors were determined using generalized linear mixed models. *Ae. (Stegomyia) spp* indices such as container index (CI) and pupae per container index (PPC) were also estimated.

*Aedes aegypti* and *Ae. bromeliae* were the most abundant vector species, accounting for 70.8% of all *Aedes* mosquitoes collected. The highest CI was observed for plastic containers irrespective of the season, whereas the highest PPC was observed for coconut shells and aluminium containers in the rainy and dry seasons, respectively. The risk of *Aedes* mosquito occurrence and abundance were significantly associated with the presence of plastic containers, coconut shells, used tyres and steel containers. These were discarded in shaded places, in the open and gardens, or found in plant nurseries.

This study shows that *Aedes* species of global health significance occur at hotel compounds on this part of Zanzibar Island. The occurrence and abundance are sustained by the presence of abundant and poorly managed solid waste and containers used for gardening tasks. This highlights an urgent need for the adoption of area-wide environmentally sustainable *Aedes* mosquito management interventions that also integrate solid waste management and ornamental plant production practices for reducing the risk of arboviral disease epidemics.

### 3.1. Background

*Aedes* mosquito species include vectors of emerging viruses that present a substantial threat to global health and socio-economic stability (Franklin et al., 2019). Dengue virus (DENV), yellow fever virus (YFV), chikungunya virus (CHIKV), and Zika virus (ZIKV) are the most notorious *Aedes*-borne viruses due to the severity of illness and magnitude of epidemics that they cause (Powell, 2016). In recent years, the African continent has reported increasing numbers of DENV, YFV and CHIKV outbreaks (Berry et al., 2018; Russo et al., 2020; Simo et al., 2019) along with increasing numbers of ZIKV cases in countries such as Angola, Guinea-Bissau, Cabo Verde and Ethiopia (WHO, 2019b). Notably, the dengue prevalence in Africa has increased dramatically over the last two decades (Simo et al., 2019), while yellow fever is showing signs of re-emerging as a public health concern after decades of considerable reduction (Gaythorpe et al., 2020; Grobbelaar et al., 2016; Ingelbeen et al., 2018). *Aedes aegypti* s.l. (henceforth *Ae. aegypti*) is the implicated vector of most explosive outbreaks (Wilder-Smith et al., 2017). However, other *Aedes* species are highly efficient at transmitting life-threatening arboviruses between humans or between other primates and humans. This includes the more poorly investigated native species, such as *Ae. bromeliae*, *Ae. africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. vittatus*, and *Ae. simpsoni* s.s (Huang, 2004). Unprecedented urbanization coupled with poor solid waste management, movement of people and commodities and, not least, climate change are considered main drivers of vector and pathogen spread (Rose et al., 2020; Russo et al., 2020; Sutherst, 2004). Moreover, it is argued that climate change could cause a shift in the burden of mosquito-borne diseases in Africa from malaria to arboviral diseases, as temperature increases may expand the environmental suitability for transmission of dengue and several other arboviral diseases on the continent (Mordecai et al., 2020).

Mainland Tanzania has observed a surge in *Aedes*-borne arboviral disease transmission, with six dengue outbreaks recorded within the past ten years. The most recent outbreak in 2019, spread from Dar-es-Salaam to Dodoma, Morogoro, Pwani, Singida and Tanga, resulting in 6,670 confirmed cases and 13 deaths (WHO, 2019a). Similarly, active co-circulation of dengue and chikungunya viruses has been reported from other regions of the mainland (Chipwaza et al., 2021a; Kajeguka et al., 2016; Kinimi et al., 2018) and neighbouring countries such as Kenya (Nyamwaya et al., 2021). Currently, there is no entomological evidence of arboviral disease transmission in the Zanzibar archipelago. However, recent serological and molecular

surveys have suggested that dengue virus may be circulating in the local population (Ali et al., 2020; Gautret et al., 2010; Shauri et al., 2021; Vairo et al., 2014). The occurrence of abundant *Ae. aegypti* and *Ae. bromeliae* across urban and rural areas of Zanzibar Island (also known as Unguja) has also been recently confirmed (Kampango et al., 2021b; Saleh et al., 2018; Saleh et al., 2020b). Infestation of the Zanzibar archipelago by these vectors is a serious threat, as arboviral epidemics will have devastating impacts on the fragile public health system and the tourism-based economy. Tourism contributes 27% of the Zanzibar GDP and 80% of foreign revenue (The Revolutionary Government of Zanzibar, 2015; 2020). However, the tourism industry is also the most important generator of poorly managed non-biodegradable solid waste in Zanzibar (The Revolutionary Government of Zanzibar, 2020; UNICEF, 2018). Some of these solid wastes can sustain a higher diversity of epidemiologically relevant mosquito taxa (Kampango et al., 2021b). In addition, hotels may concentrate visitors and staff moving to and from different highly endemic regions, which increases the likelihood of contact between local vectors and infectious hosts carrying new variants of mosquito-borne arboviruses. For instance, nearly 722,000 tourists arrived in Zanzibar archipelago between 2019 and 2020 from mainland Tanzania (Office of Chief Government Statistician-Zanzibar, 2020), considered a high-risk pathway for pathogen importation into the archipelago (Le Menach et al., 2011; Zanzibar Malaria Elimination Programme, 2009). Introduction of new variants of viruses may cause large and uncontrolled epidemics, as observed in recent outbreaks of CHIKV in Africa and Asia (Berry et al., 2018; Parola et al., 2006). A similar situation was observed for YFV in Brazil in 2018 (Cunha et al., 2019). Apart from YFV, there is not widely available, safe and effective vaccine against the main mosquito-borne viruses, nor are there any specific antiviral treatments available for the management of disease cases (Powell, 2016; Wilder-Smith et al., 2017). Therefore, vector control remains the recommended measure to limit mosquito-borne diseases (Bowman et al., 2016; Wilder-Smith et al., 2017). In Zanzibar, hotels implement periodical blanket spraying with residual effect insecticide to reduce mosquito bite exposure. This approach, apart from causing widely known environmental repercussions, such as massive decline in the biodiversity of terrestrial and aquatic non-target entomofauna, as observed elsewhere (Poulin, 2011; Sánchez-Bayo & Wyckhuys, 2019), exerts selective pressure on local vector species to develop resistance to common classes of insecticides, as recently confirmed for *Ae. aegypti* populations found at different hotels in Zanzibar (Kampango et al., 2022). This indicates that non-chemical and environmentally sustainable control practices targeting mosquito sources should be implemented to reduce the risk of arbovirus infection exposure. The design and implementation of cost-effective environmental control approaches require

accurate and thorough characterization of key environmental factors favouring the establishment and maintenance of vector populations in and around hotel settings. Hotels can be ideal locations to test the feasibility and efficacy of novel non-chemical environmental interventions before large scale implementation as they have well delimited and fully managed environments.

The main goal of this study was to determine local environment associated factors promoting occurrence and abundance of dominant vector species *Aedes aegypti* and *Ae. bromeliae* at hotel compounds. This study expands the knowledge on the entomological profile of potential arbovirus vectors occurring in Zanzibar. The findings may support the implementation of a comprehensive mosquito surveillance system and integrated arbovirus vector management since hotels in the region are not currently covered by the public surveillance system and routine vector control campaigns.

## **3.2. Material and methods**

### **3.2.1. Description of study sites**

The study was carried out at four selected hotels in the South-East coastal region of Zanzibar Island. The hotels, described in detail in Chapter 2, were selected according to compound size (total residential and non-residential area not less than one hectare), accessibility by local means of transportation during low and high tourism seasons, willingness to share data, and willingness to accept publication of findings. Consent was obtained from hotel management to conduct the study on their properties. For privacy reasons, hotel names are anonymized, and Hotel A, B, C and D used as identifiers. The largest hotel (Hotel B) occupied an area of approximately 28.06 hectares, whilst the remaining hotels encompassed an approximate area of 6.6 hectares (Hotel A), 3.6 hectares (Hotel D), and 2 hectares (Hotel C). The rainfall regime of Zanzibar is divided into two main rainy seasons, the long rainy season usually starts from mid-March to June, and the short rainy season (*Vuli*) from November to December. Average monthly precipitation ranges from 30 mm in the cold season (July) to 320 mm in the hot season (December) with the accumulated annual rainfall reaching 1600 mm (Forum, 2019a). The relative humidity is usually high, with a monthly average ranging from 87% during the long rainy season (March to June) to 76% in November and December (during the short rainy season) and reaching a minimum of 60% during the dry season (January to March and July to October) (Zanzinet Forum, 2019).

### 3.2.2. Mosquito sampling strategy

*Aedes* mosquito immature stages were sampled for a period of ten-months from September 2018 to October 2019, encompassing both the rainy and dry seasons. At each hotel, mosquitoes were surveyed over four to six days in each month using protocols proposed by Tun-Lin et al. (1994b) and Manrique-Saide et al. (2011), with small adaptations as previously detailed in Kampango et al. (2021b) and Chapter 2. For relatively small containers [water volume less than 1 litre up to 5 litres, e.g., plastic bottles and beer or soda cans (metal containers)], all larvae and pupae were sampled using pipettes. For large containers (water volume >5 litres, e.g., buckets, jerry cans and ceramic pottery) with relatively few specimens, all larvae and pupae were sampled using dippers, sweep nets or bowls. For the largest containers (water volume > 20 litres, e.g., water tanks, wells, and septic tanks), specimens were collected by 10 - 15 random dips/sweeps. If a container was too deep (e.g., wells) samples were collected using small, suspended buckets (approx. 5 litres), filling ten buckets from different sites of the water container. Containers without mosquito larvae or pupae were considered as potential larval habitats for any local mosquito species if they had water and physical integrity capable of retaining collected water for at least three consecutive days; the average time required for larvae of several Afrotropical mosquito species to hatch from eggs in the wild (Service, 1990). Larval habitats were characterized according to type, function, location, size, sun exposure, presence of vegetation, presence of organic matter, and season (Table 3.1).

**Table 3.1.** Covariates considered to model the risk factors for *Aedes* mosquito infestation and abundance at hotel compounds in Zanzibar.

| Habitat characteristics | Category                                                                                                                                                                                                                                    | Description                                                                                                                                                                                                                       |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Type                    | Plastic containers, Coconut shell, Steel container, Ceramic pot/flowerpot, Glass container, Tree hole, Concrete tank, Fibre glass container, Aluminium foil containers, Mollusc shell, Others                                               | Type of natural or man-made water holding item, artefact, utensil, etc., found with <i>Aedes</i> larvae/pupae or containing water quantity capable of sustaining mosquito immature stages (egg, larva, pupa) for at least 3 days. |
| Location                | Garden/open spaces, Plant nursery, Staff room quarter, Solid waste dumpsite/collection area, Workshop/Laundry, Kitchen area, Office/Administration area, Road/Pathway, Guest quarter, Bar/restaurant/Lobby, Sewage treatment network, Other | Geographical space within the hotel compound where mosquito larva habitats have been found.                                                                                                                                       |
| Function                | Discarded, Gardening, Cooking/washing, Decoration, Well/Water collection, Construction, AC drainage, Septic tank/Soak away, Wastewater management, Other                                                                                    | Actual daily use or purpose of the item found with mosquito larva or pupa.                                                                                                                                                        |
| Size                    | Small (< 1 m <sup>2</sup> ), medium (1 – 5 m <sup>2</sup> ), Large (> 5 m <sup>2</sup> )                                                                                                                                                    | Estimated size in square metres occupied by larval habitat                                                                                                                                                                        |
| Sun exposure            | Not exposed, exposed half a day, exposed more than half a day                                                                                                                                                                               | Amount of time the habitat has been exposed to sun light                                                                                                                                                                          |
| Organic matter*         | With organic matter, Without organic matter                                                                                                                                                                                                 | Presence of organic matter debris in the habitat                                                                                                                                                                                  |
| Vegetation              | With vegetation, Without vegetation                                                                                                                                                                                                         | Presence of floating, submerged, emerged vegetation                                                                                                                                                                               |
| Season                  | Rainy, Dry                                                                                                                                                                                                                                  | Rainy season (April – May/June; November and December); Dry season (January to March; July – October)                                                                                                                             |

\*Virtually all habitats found positive for larva/pupae contained visible organic matter. Therefore, this variable was not considered in the risk factor modelling as its association with mosquito was obvious.

### 3.2.3. Sample processing and identification

Samples of immature mosquitoes were reared at insectary environmental conditions of  $27 \pm 2^\circ\text{C}$  and  $75 \pm 10\%$  relative humidity until they emerged as adults (WHO, 2016). Larvae were fed ground adult cat food biscuits and kept in a 12:12h light:dark regime until adult emergence. Adult mosquitoes were euthanised by placing the specimens inside a refrigerator. Mosquitoes were identified morphologically down to species levels using taxonomic keys for Afrotropical mosquito fauna (Huang, 2004; Jupp, 1986). Mosquitoes that could not be identified morphologically to species, such as members of species complexes, were placed individually in Eppendorf tubes with silica gel and store at  $-20^\circ\text{C}$  for further molecular analysis.

### 3.3. Statistical analysis

#### 3.3.1. *Aedes* entomological indices

The following entomological indicators for *Aedes (Stegomyia) spp.* were estimated: container index (CI), pupae per container (PPC) and proportion of positive containers (PC). CI was estimated by dividing the total number of positive containers of a given type against the total number of all inspected containers. The PPC was estimated by dividing the total number of pupae collected from a given type of container by the total number of containers inspected, whereas the PC index was estimated as the quotient between the total number of a type of positive containers by the total number of all positive containers found.

#### 3.3.2. Modelling the risk factors for *Aedes* mosquito

Mosquito immature counts had excess zeros as the overall proportion of zero counts was over 30%. The counts were also over-dispersed, showing a positive mean–variance relationship (Hilbe, 2011). Therefore, we applied zero-inflated negative binomial mixed models (ZINBM) with *log* link function, to determine local factors contributing to the abundance of *Ae. aegypti* and *Ae. bromeliae* at study sites, while accounting for excess zeros and over-dispersion (Hilbe, 2014). Dependent variables were *Ae. aegypti* and *Ae. bromeliae* counts. On the other hand, mixed effect logistic regression was applied to determine the risk factor for presence of any *Aedes* species in general. The dependent variable was the presence of *Aedes* immatures (larvae and pupae) (Yes=1, No=0). For this case, we assumed that the distribution of presence and absence of *Aedes* immature records followed a binomial distribution with *logit* function. For ZINBM and mixed effect logistic regression models the potential risk factors considered were

type of container, location, function, size, sun exposure, presence of vegetation and season (Table 3.1). Organic matter was always present in containers with immature *Aedes*, so it was not included in the model. Hotel and day of survey were considered random factors to account for dependence between repeated measurements across sites over time. A likelihood ratio test comparing the null model (model with dependent variable only) and model with the random factor was applied to determine whether adding a random factor was justifiable. Multi-collinearity between predictors was evaluated using the variance inflation factor (VIF). VIF estimates less than or equal to three indicated low collinearity (Zuur et al., 2010). Variables showing high collinearity were fitted separately with other uncorrelated predictors. Model distributional assumptions were determined by examining model residuals for normality, dispersion, and independence (no autocorrelation among residuals). Models were fitted using the package glmmTMB v. 1.0.2.9. (Brooks et al., 2017). To deal with convergence problems, we combined all underrepresented levels of predictor variables, comprising less than 1% of all level observations, and denoted them as "Other". We assumed that these underrepresented levels would introduce more information to the model combined rather than alone. Model diagnostics were performed using the package DHARMa v. 0.3.3.0 (Hartig, 2020). Multi-collinearity between covariates was determined using the package performance v.0.6.1 (Lüdtke et al., 2020). All data processing tasks and analysis were performed via the software R v. 4.2.0 (R Core Team, 2022).

### 3.4. Results

#### 3.4.1. *Aedes* populations and larval habitats

A total of 1,466 water-holding containers were inspected out of which, 763 were found positive for *Aedes* mosquitoes (larvae and pupae). A total of 19,533 *Aedes* mosquitoes were collected, comprising 16,187 larvae and 3,346 pupae. Of these, 83% ( $n = 16,207$ ) and 15.6% ( $n = 3,043$ ) were *Aedes* (*Stegomyia*) *aegypti* and *Ae.* (*Stegomyia*) *bromeliae*, respectively. Other less frequent species; *Ae.* (*Stegomyia*) *vittatus*, *Ae.* (*Stegomyia*) *africanus*, *Ae.* (*Stegomyia*) *metallicus*, *Ae.* (*Stegomyia*) *unilineatus*, *Ae.* (*Stegomyia*) *calceatus*, *Ae.* (*Stegomyia*) *heischii* and *Ae.* (*Pseudarmigeress*) *natalensis*, comprised 1.4% ( $n = 283$ ) of all specimens sampled (Table 3.2 and Table 3.3). Plastic containers (mostly bottles and yoghurt cups) were by far the most frequent type of habitat found with larvae and/or pupae. They comprised 69.9% ( $n = 372$ ) and 71.4% ( $n = 165$ ) of all positive habitats recorded in the rainy and dry seasons, respectively, followed by coconut shells 7.4% ( $n = 38$ ) vs 3.4% ( $n = 8$ ), metal containers 4.7% ( $n = 25$ ) vs

4.3% (n = 10) and used tyres 4.5% (n = 24) vs 1.7% (n = 4). Nearly 83.4% (n = 1,223) of all water holding containers inspected were discarded items, followed by containers used for gardening activities (5.3%; n = 78; e.g., cut jerry cans and plastic bottles for plant propagation (Table 3.2 and Figure 3.1). Containers were mostly at gardens or open spaces (62.1%; n = 909), staff quarters (11.6%; n = 170), plant nurseries (7.6%; n = 112) and solid waste dumpsite areas (4.2%; n = 62) (Table A3.1).

The highest relative container index (CI) was found for plastic containers irrespective of the season. The density of pupae per container (PPC) was highest for steel containers (PPC = 5.42) and coconut shells (PPC = 5.38) in the rainy season. In the dry season, the highest PPC was observed for aluminium containers (PPC = 10.0) and used tyres (PPC = 5.10) (Table 3.3)

**Table 3.2.** Relative abundance of *Aedes* mosquito at hotel compounds stratified according to larval habitat characteristics, that is, type of habitat, location, function, size, presence of vegetation, presence of organic matter and season.

| Variable                 | Larva habitats |              | Aedes mosquito abundance |                |                     |                          |                            |                                |
|--------------------------|----------------|--------------|--------------------------|----------------|---------------------|--------------------------|----------------------------|--------------------------------|
|                          | Inspected (%)  | Positive (%) | Total larva (%)          | Total pupa (%) | Total mosquito (%)* | <i>Aedes aegypti</i> (%) | <i>Aedes bromeliae</i> (%) | Other <i>Aedes</i> species (%) |
| <b>Type of habitat</b>   |                |              |                          |                |                     |                          |                            |                                |
| Plastic container        | 1026 (69.99)   | 537 (70.38)  | 12131 (62.11)            | 2463 (73.61)   | 14594 (63.81)       | 11622 (71.71)            | 2738 (89.98)               | 234 (82.69)                    |
| Coconut shell            | 77 (5.25)      | 46 (6.03)    | 1275 (6.53)              | 306 (9.15)     | 1581 (6.91)         | 1517 (9.36)              | 64 (2.1)                   | 0 (0)                          |
| Used tyre                | 40 (2.73)      | 28 (3.67)    | 808 (4.14)               | 168 (5.02)     | 976 (4.27)          | 966 (5.96)               | 10 (0.33)                  | 0 (0)                          |
| Steel container          | 64 (4.37)      | 35 (4.59)    | 730 (3.74)               | 206 (6.16)     | 936 (4.09)          | 812 (5.01)               | 103 (3.38)                 | 21 (7.42)                      |
| Ceramic pot/flowerpot    | 31 (2.11)      | 21 (2.75)    | 234 (1.2)                | 47 (1.4)       | 281 (1.23)          | 249 (1.54)               | 31 (1.02)                  | 1 (0.35)                       |
| Glass container          | 46 (3.14)      | 14 (1.83)    | 206 (1.05)               | 8 (0.24)       | 214 (0.94)          | 165 (1.02)               | 34 (1.12)                  | 15 (5.3)                       |
| Tree hole                | 15 (1.02)      | 9 (1.18)     | 126 (0.65)               | 13 (0.39)      | 139 (0.61)          | 129 (0.8)                | 2 (0.07)                   | 8 (2.83)                       |
| Concrete tank            | 79 (5.39)      | 36 (4.72)    | 104 (0.53)               | 21 (0.63)      | 125 (0.55)          | 125 (0.77)               | 0 (0)                      | 0 (0)                          |
| Fibre glass container    | 4 (0.27)       | 3 (0.39)     | 87 (0.45)                | 1 (0.03)       | 88 (0.38)           | 88 (0.54)                | 0 (0)                      | 0 (0)                          |
| Aluminium foil container | 6 (0.41)       | 3 (0.39)     | 71 (0.36)                | 10 (0.3)       | 81 (0.35)           | 54 (0.33)                | 23 (0.76)                  | 4 (1.41)                       |
| Mollusc shell            | 7 (0.48)       | 4 (0.52)     | 22 (0.11)                | 4 (0.12)       | 26 (0.11)           | 23 (0.14)                | 3 (0.1)                    | 0 (0)                          |
| Other                    | 71 (4.84)      | 27 (3.54)    | 393 (2.01)               | 99 (2.96)      | 492 (2.15)          | 457 (2.82)               | 35 (1.15)                  | 0 (0)                          |
| <b>Location</b>          |                |              |                          |                |                     |                          |                            |                                |
| Garden/Open spaces       | 909 (62.01)    | 453 (59.37)  | 12140 (62.15)            | 2123 (63.45)   | 12140 (53.08)       | 9461 (58.38)             | 2459 (80.81)               | 220 (77.74)                    |
| Plant nursery            | 112 (7.64)     | 74 (9.7)     | 2360 (12.08)             | 490 (14.64)    | 2360 (10.32)        | 2083 (12.85)             | 240 (7.89)                 | 37 (13.07)                     |
| Staff quarter            | 170 (11.6)     | 81 (10.62)   | 1574 (8.06)              | 184 (5.5)      | 1574 (6.88)         | 1505 (9.29)              | 63 (2.07)                  | 6 (2.12)                       |
| Solid waste management   | 62 (4.23)      | 42 (5.5)     | 704 (3.6)                | 116 (3.47)     | 704 (3.08)          | 596 (3.68)               | 108 (3.55)                 | 0 (0)                          |
| Workshop/laundry area    | 42 (2.86)      | 19 (2.49)    | 699 (3.58)               | 137 (4.09)     | 699 (3.06)          | 601 (3.71)               | 97 (3.19)                  | 1 (0.35)                       |
| Kitchen area             | 25 (1.71)      | 14 (1.83)    | 468 (2.4)                | 45 (1.34)      | 468 (2.05)          | 449 (2.77)               | 13 (0.43)                  | 6 (2.12)                       |

|                               |              |             |               |              |               |               |              |             |
|-------------------------------|--------------|-------------|---------------|--------------|---------------|---------------|--------------|-------------|
| Office/Administration         | 15 (1.02)    | 10 (1.31)   | 446 (2.28)    | 52 (1.55)    | 446 (1.95)    | 433 (2.67)    | 10 (0.33)    | 3 (1.06)    |
| Road/Street/Pathway           | 37 (2.52)    | 23 (3.01)   | 378 (1.94)    | 84 (2.51)    | 378 (1.65)    | 346 (2.13)    | 26 (0.85)    | 6 (2.12)    |
| Guest room area               | 43 (2.93)    | 23 (3.01)   | 342 (1.75)    | 75 (2.24)    | 342 (1.5)     | 336 (2.07)    | 2 (0.07)     | 4 (1.41)    |
| Bar/Restaurante area          | 6 (0.41)     | 3 (0.39)    | 82 (0.42)     | 5 (0.15)     | 82 (0.36)     | 82 (0.51)     | 0 (0)        | 0 (0)       |
| Sewage treatment network      | 6 (0.41)     | 4 (0.52)    | 6 (0.03)      | 0 (0)        | 6 (0.03)      | 5 (0.03)      | 1 (0.03)     | 0 (0)       |
| Other                         | 39 (2.66)    | 17 (2.23)   | 334 (1.71)    | 35 (1.05)    | 334 (1.46)    | 310 (1.91)    | 24 (0.79)    | 0 (0)       |
| <b>Function</b>               |              |             |               |              |               |               |              |             |
| Discarded                     | 1223 (83.42) | 628 (82.31) | 16220 (83.04) | 2515 (75.16) | 16220 (70.92) | 13083 (80.72) | 2863 (94.08) | 274 (96.82) |
| Gardening                     | 78 (5.32)    | 51 (6.68)   | 2063 (10.56)  | 622 (18.59)  | 2063 (9.02)   | 1950 (12.03)  | 105 (3.45)   | 8 (2.83)    |
| Cooking/Washing               | 19 (1.3)     | 10 (1.31)   | 133 (0.68)    | 12 (0.36)    | 133 (0.58)    | 116 (0.72)    | 17 (0.56)    | 0 (0)       |
| Decoration                    | 6 (0.41)     | 5 (0.66)    | 119 (0.61)    | 33 (0.99)    | 119 (0.52)    | 117 (0.72)    | 1 (0.03)     | 1 (0.35)    |
| Well/Rain water collection    | 19 (1.3)     | 9 (1.18)    | 40 (0.2)      | 2 (0.06)     | 40 (0.17)     | 40 (0.25)     | 0 (0)        | 0 (0)       |
| Construction                  | 6 (0.41)     | 3 (0.39)    | 25 (0.13)     | 2 (0.06)     | 25 (0.11)     | 25 (0.15)     | 0 (0)        | 0 (0)       |
| AC drainage                   | 6 (0.41)     | 1 (0.13)    | 30 (0.15)     | 0 (0)        | 30 (0.13)     | 30 (0.19)     | 0 (0)        | 0 (0)       |
| Septic tank/Soak away         | 40 (2.73)    | 17 (2.23)   | 26 (0.13)     | 0 (0)        | 26 (0.11)     | 26 (0.16)     | 0 (0)        | 0 (0)       |
| Wastewater management         | 4 (0.27)     | 2 (0.26)    | 29 (0.15)     | 19 (0.57)    | 29 (0.13)     | 29 (0.18)     | 0 (0)        | 0 (0)       |
| Other                         | 65 (4.43)    | 37 (4.85)   | 848 (4.34)    | 141 (4.21)   | 848 (3.71)    | 791 (4.88)    | 57 (1.87)    | 0 (0)       |
| <b>Container size</b>         |              |             |               |              |               |               |              |             |
| Small (<1m <sup>2</sup> )     | 1365 (93.11) | 711 (93.18) | 19277 (98.69) | 3287 (98.24) | 19277 (84.28) | 15973 (98.56) | 3021 (99.28) | 283 (100)   |
| Medium (1 - 5m <sup>2</sup> ) | 69 (4.71)    | 38 (4.98)   | 225 (1.15)    | 59 (1.76)    | 225 (0.98)    | 203 (1.25)    | 22 (0.72)    | 0 (0)       |
| Large (>5m <sup>2</sup> )     | 32 (2.18)    | 14 (1.83)   | 31 (0.16)     | 0 (0)        | 31 (0.14)     | 31 (0.19)     | 0 (0)        | 0 (0)       |
| <b>Sun exposure</b>           |              |             |               |              |               |               |              |             |
| Half a day                    | 1320 (90.04) | 718 (94.1)  | 19050 (97.53) | 3302 (98.68) | 19050 (83.29) | 15738 (97.11) | 3029 (99.54) | 283 (100)   |
| More than half a day          | 108 (7.37)   | 30 (3.93)   | 421 (2.16)    | 44 (1.32)    | 421 (1.84)    | 409 (2.52)    | 12 (0.39)    | 0 (0)       |
| Not exposed                   | 38 (2.59)    | 15 (1.97)   | 62 (0.32)     | 0 (0)        | 62 (0.27)     | 60 (0.37)     | 2 (0.07)     | 0 (0)       |
| <b>Organic matter</b>         |              |             |               |              |               |               |              |             |
| With Organic matter           | 1459 (99.52) | 763 (100)   | 19533 (100)   | 3346 (100)   | 19533 (85.4)  | 16207 (100)   | 3043 (100)   | 283 (100)   |

|                               |              |             |               |              |               |               |              |             |
|-------------------------------|--------------|-------------|---------------|--------------|---------------|---------------|--------------|-------------|
| Without Organic matter        | 7 (0.48)     | 0 (0)       | 0 (0)         | 0 (0)        | 0 (0)         | 0 (0)         | 0 (0)        | 0 (0)       |
| <b>Presence of vegetation</b> |              |             |               |              |               |               |              |             |
| With vegetation               | 122 (8.32)   | 79 (10.35)  | 2017 (10.33)  | 122 (3.65)   | 2017 (8.82)   | 1403 (8.66)   | 585 (19.22)  | 29 (10.25)  |
| Without vegetation            | 1344 (91.68) | 684 (89.65) | 17516 (89.67) | 3224 (96.35) | 17516 (76.58) | 14804 (91.34) | 2458 (80.78) | 254 (89.75) |
| <b>Season</b>                 |              |             |               |              |               |               |              |             |
| Rainy                         | 814 (55.53)  | 532 (69.72) | 14177 (72.58) | 2355 (70.38) | 14177 (61.98) | 12042 (74.3)  | 2057 (67.6)  | 78 (27.56)  |
| Dry                           | 652 (44.47)  | 231 (30.28) | 5356 (27.42)  | 991 (29.62)  | 5356 (23.42)  | 4165 (25.7)   | 986 (32.4)   | 205 (72.44) |

\*Sum of total larva and total pupa collected



**Figure 3.1.** Common sources of *Aedes* mosquitoes found at hotel compounds on the south-eastern coastal region of Zanzibar Island. Flowerpot (a); coconut shell (b); discarded tyre (c); cement tank (d); tree hollow (e); food can (f); water bottle (g), mollusc shell (h); cut jerry cans and water bottles with rooting plants (i), glass bottle (j); coconut flower spathe (k), aluminium container (l).

**Table 3.3.** Composition and relative productivity of larval habitats found positive for *Aedes* mosquito at hotel compound on Zanzibar Island.

| Season | Type of container        | Number of containers |              | Stegomyia larva index |       |       | Aedes abundance |            |               |                 |             |
|--------|--------------------------|----------------------|--------------|-----------------------|-------|-------|-----------------|------------|---------------|-----------------|-------------|
|        |                          | Inspected (%)        | Positive (%) | CI                    | PC    | PPC   | Aedes larva     | Aedes pupa | Aedes aegypti | Aedes bromeliae | Other Aedes |
| Rainy  | Plastic container        | 571 (70.1)           | 372 (69.9)   | 0.46                  | 0.70  | 2.88  | 8,551           | 1,644      | 8,319         | 1,803           | 73          |
|        | Coconut shell            | 56 (6.9)             | 38 (7.1)     | 0.05                  | 0.07  | 5.38  | 1,260           | 301        | 1,497         | 64              | 0           |
|        | Used tyre                | 30 (3.7)             | 24 (4.5)     | 0.03                  | 0.05  | 3.90  | 665             | 117        | 772           | 10              | 0           |
|        | Steel container          | 33 (4.1)             | 25 (4.7)     | 0.03                  | 0.05  | 5.42  | 547             | 179        | 669           | 57              | 0           |
|        | Ceramic pot/flowerpot    | 19 (2.3)             | 17 (3.2)     | 0.02                  | 0.03  | 2.42  | 220             | 46         | 235           | 30              | 1           |
|        | Glass container          | 23 (2.8)             | 13 (2.4)     | 0.02                  | 0.02  | 0.35  | 191             | 8          | 165           | 34              | 0           |
|        | Tree hole                | 5 (0.6)              | 4 (0.8)      | 0.00                  | 0.01  | 2.60  | 72              | 13         | 84            | 1               | 0           |
|        | Concrete tank            | 31 (3.8)             | 14 (2.6)     | 0.02                  | 0.03  | 0.00  | 50              | 0          | 50            | 0               | 0           |
|        | Aluminium foil container | 5 (0.6)              | 2 (0.4)      | 0.002                 | 0.004 | 0.00  | 62              | 0          | 35            | 23              | 4           |
|        | Mollusc shell            | 5 (0.6)              | 4 (0.8)      | 0.005                 | 0.01  | 0.80  | 22              | 4          | 23            | 3               | 0           |
|        | Other                    | 36 (4.4)             | 19 (3.6)     | 0.02                  | 0.04  | 1.19  | 182             | 43         | 193           | 32              | 0           |
| Dry    | Plastic container        | 455 (69.8)           | 165 (71.4)   | 0.25                  | 0.51  | 1.80  | 3,580           | 819        | 3,303         | 935             | 161         |
|        | Coconut shell            | 21 (3.2)             | 8 (3.5)      | 0.01                  | 0.02  | 0.24  | 15              | 5          | 20            | 0               | 0           |
|        | Used tyre                | 10 (1.5)             | 4 (1.7)      | 0.01                  | 0.01  | 5.10  | 143             | 51         | 194           | 0               | 0           |
|        | Steel container          | 31 (4.8)             | 10 (4.3)     | 0.02                  | 0.03  | 0.87  | 183             | 27         | 143           | 46              | 21          |
|        | Ceramic pot/flowerpot    | 12 (1.8)             | 4 (1.7)      | 0.01                  | 0.01  | 0.08  | 14              | 1          | 14            | 1               | 0           |
|        | Glass container          | 23 (3.5)             | 1 (0.4)      | 0.002                 | 0.00  | 0.00  | 15              | 0          | 0             | 0               | 15          |
|        | Tree hole                | 10 (1.5)             | 5 (2.2)      | 0.01                  | 0.02  | 0.00  | 54              | 0          | 45            | 1               | 8           |
|        | Concrete tank            | 48 (7.4)             | 22 (9.5)     | 0.03                  | 0.07  | 0.44  | 54              | 21         | 75            | 0               | 0           |
|        | Fibre glass container    | 4 (0.6)              | 3 (1.3)      | 0.005                 | 0.01  | 0.25  | 87              | 1          | 88            | 0               | 0           |
|        | Aluminium foil container | 1 (0.2)              | 1 (0.4)      | 0.002                 | 0.003 | 10.00 | 9               | 10         | 19            | 0               | 0           |
|        | Mollusc shell            | 2 (0.3)              |              |                       |       |       |                 |            |               |                 |             |
|        | Other                    | 35 (5.4)             | 8 (3.5)      | 0.01                  |       | 1.60  | 211             | 56         | 264           | 3               | 0           |

CI = Container index, PC = Proportion of positive container, PPC = Pupa per container

### 3.4.2. Risk factors for occurrence of *Aedes* mosquitoes

The mixed effects logistic regression model indicated that natural and artificial containers such as used tyres, steel containers, plastic containers (e.g., water bottles, jerry cans) and coconut shells were more likely to harbour at least one immature stage of any of the identified *Aedes* mosquitoes compared to other types of water holding containers (Figure 3.2). The likelihood of immature mosquito occurrence was not statistically different between locations within the compounds. It also did not differ according to container function or purpose. Additionally, containers of medium size and containers that were less exposed to sunlight were less likely to be infested than larger containers or those receiving sunlight for more than half a day (Figure 3.2).

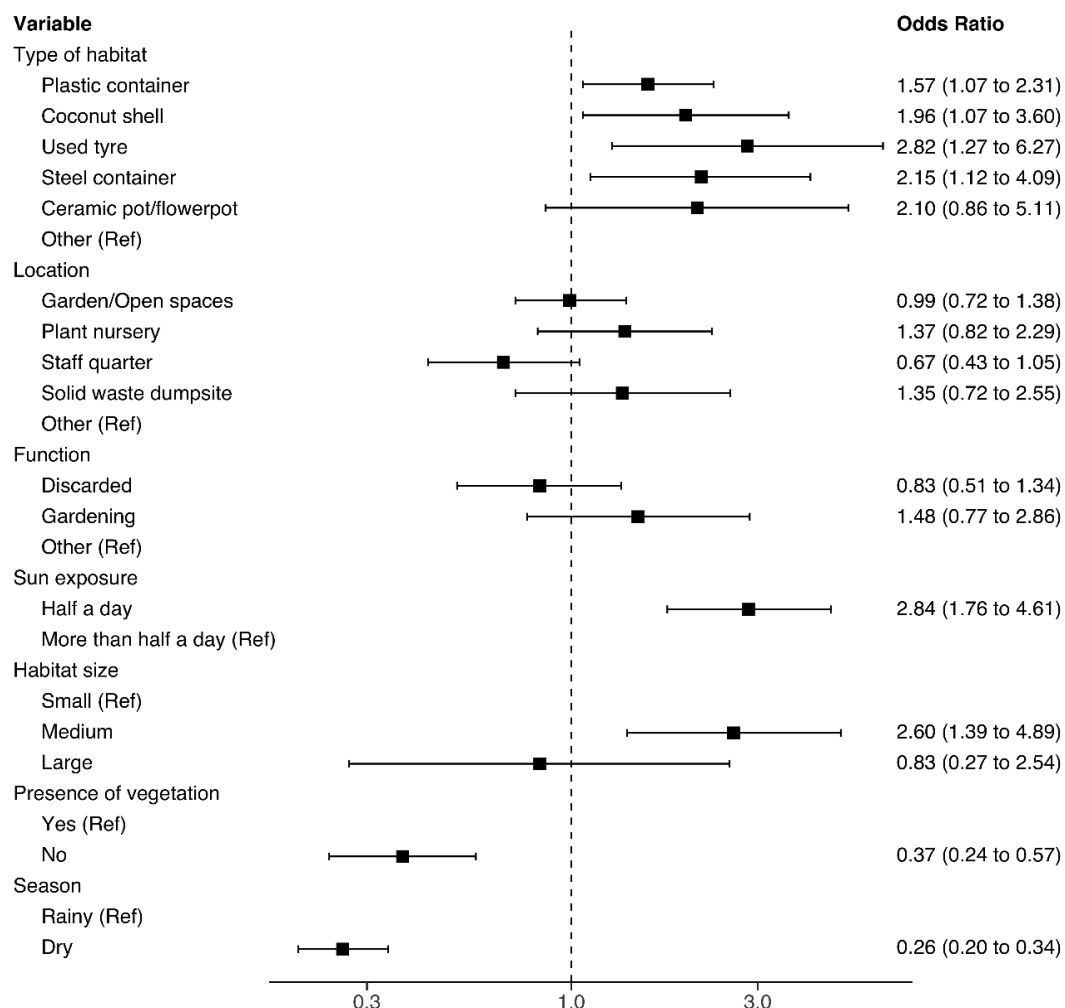


**Figure 3.2.** Results from a mixed effects logistic regression model showing the contribution of environment-associated factors for the risk of occurrence of *Aedes* mosquitoes at hotels compounds on the south-eastern coastal region of Zanzibar Island.

### 3.4.3. Risk factors for abundance of *Aedes aegypti* and *Aedes bromeliae*

Figure 3.3 indicates the abundance of *Ae. aegypti* was, in order of magnitude, significantly associated with coconut shells, plastic containers, used tyres and steel containers when compared with other types of containers. The abundance was significantly higher at plant nurseries and garden/open spaces compared to other locations inside the premises. Larval habitats associated with the highest *Ae. aegypti* abundance were mostly discarded items and containers used in gardening tasks and exposed to sunlight for less than half of the day (Figure 3.3). Habitats with and without vegetation produced statistically comparable mosquito abundances. In contrast, medium and large sized containers as well as the dry season were associated with lower abundance of *Ae. aegypti* (Figure 3.3).

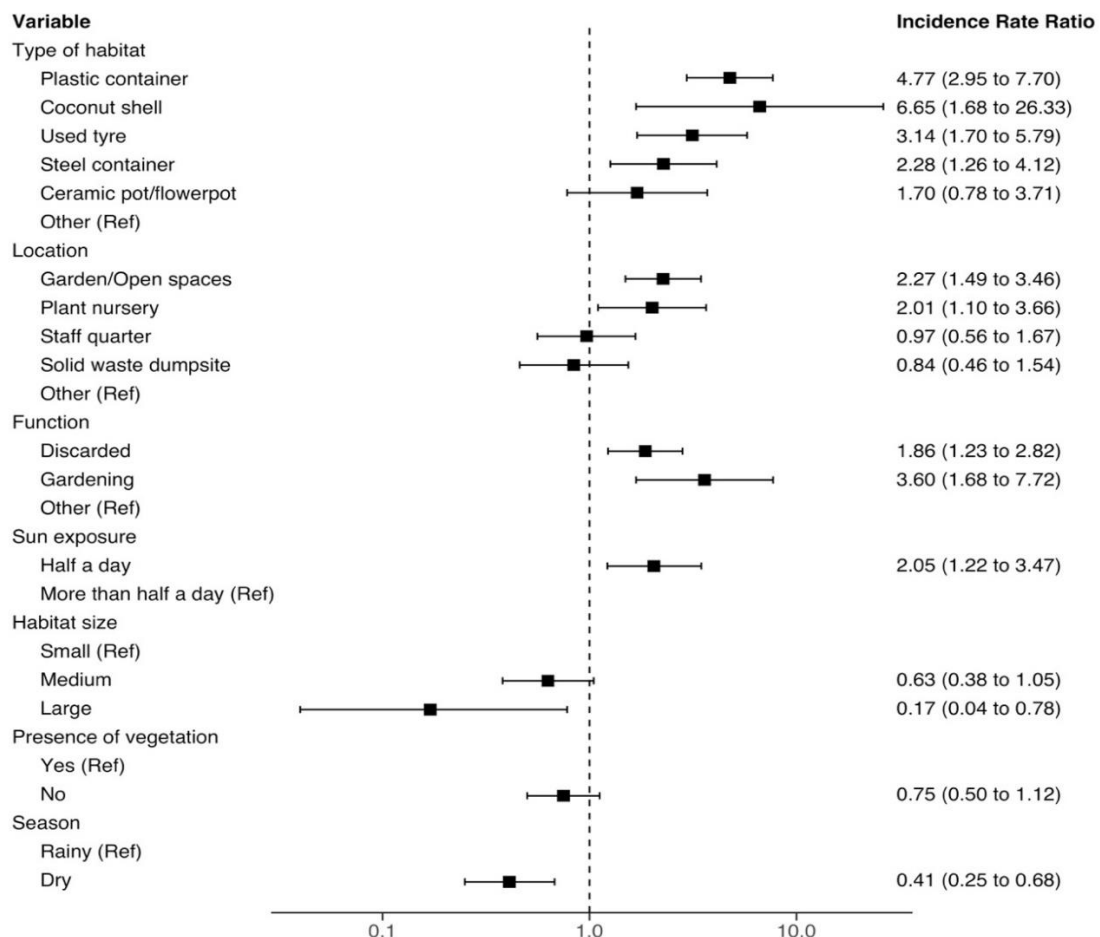


**Figure 3.3.** Results from a zero-inflated negative binomial mixed effects regression model showing the contribution of environment-associated factors for abundance of *Aedes aegypti* at hotels compounds on the south-eastern coastal region of Zanzibar Island.

Equally, *Ae. bromeliae* was highly abundant in discarded plastic containers found in gardens/open spaces and plant nurseries, and those exposed to sunlight for nearly half of the day (Figure 3.4).

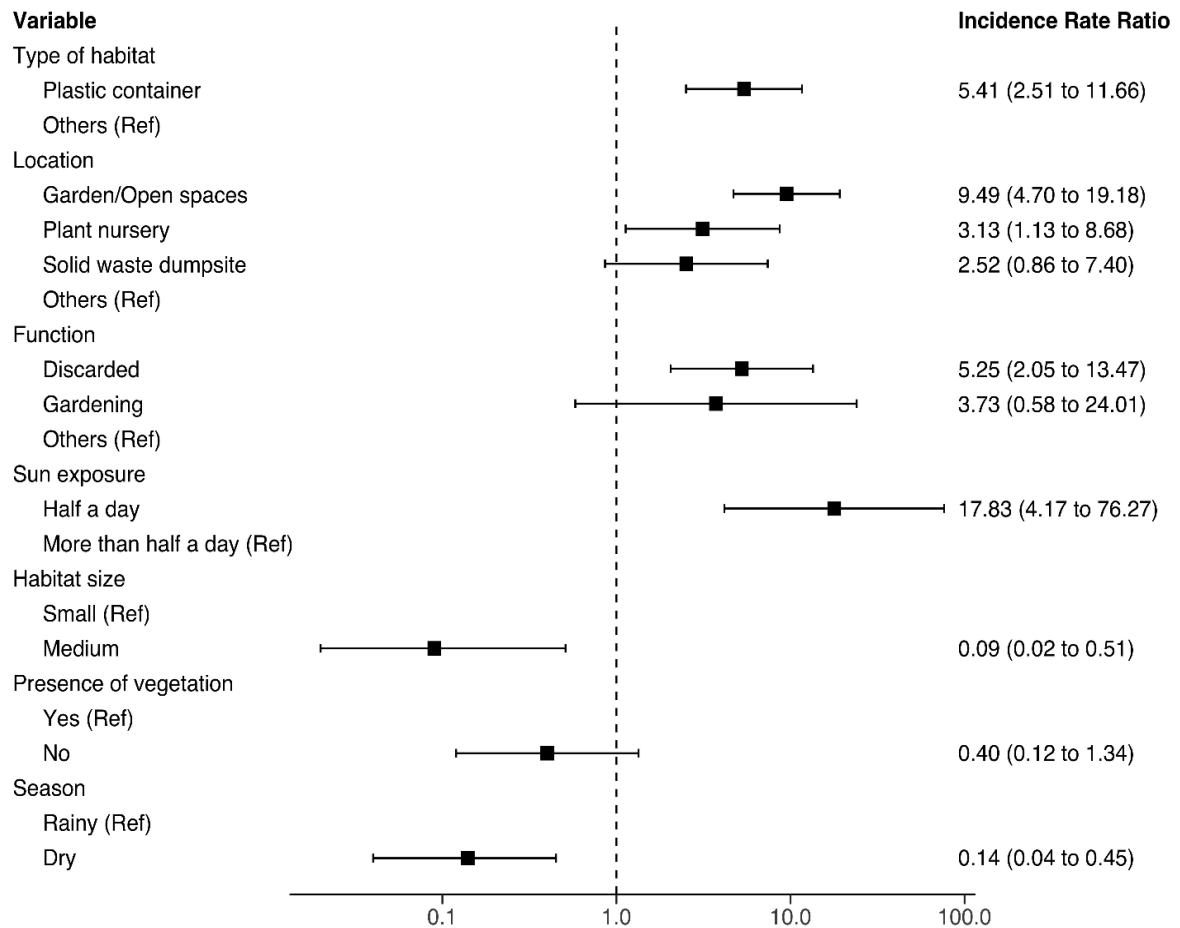


**Figure 3.4.** Results from a zero-inflated negative binomial mixed effects regression model showing the contribution of environment-associated factors for abundance of *Aedes bromeliae* at hotels compounds on the south-eastern coastal region of Zanzibar Island.

### 3.5. Discussion

Understanding the contribution of environmental factors promoting occurrence and abundance of *Aedes* mosquitoes across space and time is pivotal for the design of cost-effective sustainable vector control interventions. This could involve the raising of local awareness about solid waste disposal to reduce the risk of mosquito-borne disease exposure and epidemics. Our data clearly indicate that some hotel compounds in Zanzibar provide suitable environmental conditions for establishment of *Aedes aegypti* and *Ae. bromeliae*, and other less frequent *Aedes* mosquito vector species, such as *Ae. vittatus*, *Ae. africanus* and *Ae. metallicus*. We found that occurrence and abundance of these vector species are significantly associated with the presence of improperly managed natural and artificial solid wastes and ornamental containers. A great number of studies have reported strong associations between the abundance of *Aedes* mosquitoes and their larval habitats and poorly managed disposables as well as decorative containers (Abe et al., 2005; Banerjee et al., 2013; Zahouli et al., 2017). These materials can hold water and organic debris for long periods, providing relatively stable micro-environmental and micro-climatic conditions for proliferation of diverse mosquito species (Saleh et al., 2018; Saleh et al., 2020b; Tedjou et al., 2020; Zahouli et al., 2017). We also found high productivity of *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. bromeliae*, in natural and artificial containers such as coconut shells, plastic containers, steel containers and used tyres. These types of habitats contributed 92.6% and 93.9% of all *Aedes* larvae and pupae collected, respectively. This finding somewhat corroborates those of Saleh et al. (Saleh et al., 2018; Saleh et al., 2020b) who reported higher risk of *Ae. aegypti* exposure associated with plastic containers and used tyres across rural and urban communities in Zanzibar. However, in contrast with Saleh et al. (Saleh et al., 2018; Saleh et al., 2020b), concrete tanks were one of the least productive habitats in our study sites, contributing only 0.64% of all mosquitoes collected. Plastic containers had among the low density of *Aedes* pupae per container inspected despite being the type of containers with the highest positivity rate for *Aedes* mosquito immature stages. This surprising finding may have several interpretations. For instance, it implies that plastic containers may be less suitable for sustaining the development of *Aedes* mosquito larvae to the pupal stage. The finding may also suggest that the development rate of *Aedes* larval stages into pupae is slower in plastic containers compared to other type of containers. However, it is worth also noting that the development rate and survival rate of mosquitoes at larval stages is a density-dependence process (Walker et al., 2021). Several studies have

showed that development and survival rates of *Ae. aegypti* and *Ae. albopictus* are reduced in overpopulated larval containers (Briegel & Timmermann, 2001; Couret et al., 2014). Food availability in the larval containers is another limiting factor for larval progression between developmental stages and survival rate. For *Ae. aegypti* reared under conditions of food shortage, development and survival rate is significantly constrained (Couret et al., 2014), which may affect the likelihood of detecting pupae. Additional studies with *Ae. aegypti* have also reported lower productivity for plastic than other types of containers (Flaibani et al., 2020; Mukhtar et al., 2018). Moreover, variation of physicochemical characteristics of the contained water can also affect larval stages detection, as these factors influence both development and survival of mosquitoes at earlier stages. For instance, it has been shown that the average diurnal water temperature in plastic containers can reach up to 35.6 °C, exceeding the thermal optima range of 15°C - 35°C for the development of *Ae. aegypti* immatures (Kumar et al., 2018a; Tun-Lin et al., 2000). Therefore, plastic containers may become unsuitable larval sites depending on their daily amount of sunlight exposure. On the other hand, higher levels of pH and salinity may also be limiting factors (Medeiros-Sousa et al., 2020). However, Saleh et al. (2018) found no clear association between variations of physicochemical factors and presence of *Ae. aegypti* in Zanzibar City. Further studies on ecological and physiological determinants of the occurrence and abundance of *Aedes* mosquito immature stages in larval habitats are encouraged.

Mixed effects logistic regression and zero-inflated negative binomial mixed models revealed used tyres, plastic containers, coconut shells and metal containers (e.g., beer, soda, and food cans) as the types of containers most associated with higher risk of *Ae. aegypti* and *Ae. bromeliae* presence and abundance, respectively. However, the likelihood of infestation was comparable between locations and type of container function or purpose. In contrast with the risk of infestation, the overall abundance of *Aedes* mosquitoes was up to nine times higher (e.g., *Ae. bromeliae*) in gardens/open spaces and plant nurseries compared to other locations. This finding suggests that these locations should be prioritized when implementing mosquito control interventions. Moreover, the result also indicates that mosquito management practices should be incorporated into ornamental plant production and trading activities as it may contribute tremendously to maintenance and rapid dissemination of native and invasive vector species throughout the archipelago. Notably, invasive vector species such as *Ae. albopictus* have been successfully introduced and spread worldwide by the trade of ornamental flowers (Lounibos, 2002; Madon et al., 2002). Higher

mosquito abundance was consistently associated with discarded materials and containers used in gardening activities than for other purposes and/or functions. Similar findings were reported for community settings in Zanzibar (Saleh et al., 2020b) and Argentina (Flaibani et al., 2020). Furthermore, containers less exposed to sunlight, usually found in densely vegetated places, were more likely to be infested and to have higher mosquito abundance. Similar findings have been reported in rural and urban areas of Zanzibar and elsewhere, suggesting that targeted environmental manipulation of shaded places should be prioritized in efforts to control *Aedes* mosquitoes (Kumar et al., 2018a; Saleh et al., 2020a). Virtually, all containers found positive for either *Aedes* larvae or pupae contained organic matter detritus. It is well documented that dissolved organic matter provides conditions for development of microorganisms that can act as food for mosquito larvae. However, most recent evidence also shows that dissolved organic matter plays a key role in protecting mosquito immature stages from the lethal effects of solar ultraviolet radiation (Berry et al., 2020).

The association between poorly managed solid waste and high risk of *Aedes* infestation and abundance in public (Saleh et al., 2018; Saleh et al., 2020b) and private settings (this study) provides evidence for the design and implementation of sustainable environmental mosquito control practices. This includes integrated and consistent solid waste management strategies, periodical inspection and substitution and drainage of ornamental plant water and periodical waste removal campaigns, to reduce the risk of *Aedes*-borne arbovirus epidemics. Taking these actions is essential given recent evidence of resistance in *Ae. aegypti* from Zanzibar to some classes of insecticides approved for use in public health (Kampango et al., 2022) (Chapter 4). Additionally, this finding is also a call for implementation of strong legislation and regulation leading to environmentally safe waste disposal and treatment. This study was carried out in a relatively small number of hotels, which prevents us from making broader generalizations. Similar types of investigations involving hotels in other eco-geographical regions of Zanzibar may provide a more comprehensive understanding of risks factors for mosquito-borne arbovirus exposure at hotels in the archipelago.

### 3.6. Conclusion

We conclude that unmanaged solid waste from artificial and natural sources is the most important risk factor for the occurrence and abundance of the arbovirus vectors *Aedes aegypti* and *Ae. bromeliae* at hotel compounds investigated in this study. Notably, the most important sources of *Aedes* mosquitoes are disposable plastic containers (water bottles, yoghurt cups, jerry cans, etc), used tyres and metal containers (e.g., soda, beer, and food cans) as well as natural products such as coconut shells. Future vector control interventions should target these types of larval habitats, especially in shaded places in the garden areas, plant nurseries and in the surrounding communities. Findings from this study underscore the need for development and implementation of area-wide mosquito control interventions that incorporate waste management practices, vegetation manipulation and community mobilization for environmental sanitation. Hotels present a unique opportunity to test the cost-effectiveness of these integrated control approaches, given the human resources, the financial incentives and structured/systematic management of the compounds. We propose that future eco-certification/classification of tourism facilities could include implementation of area-wide non-chemical environmental management practices to reduce mosquito proliferation as an indicator, since mosquito infestation rates remain high in spite of periodic mosquito control activities involving use of chemical insecticides.

### 3.7. Acknowledgements

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## CHAPTER 4

### **High DDT resistance without apparent association to *kdr* and Glutathione-S-transferase (GST) gene mutations in *Aedes aegypti* population at hotel compounds in Zanzibar**

This chapter addresses objective five and six, and it has been published with slight modification as:

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## Abstract

Global efforts to control *Aedes* mosquito-transmitted pathogens still rely heavily on insecticides. However, available information on vector resistance is mainly restricted to mosquito populations located in residential and public areas, whereas commercial settings, such as hotels are overlooked. This may obscure the real magnitude of the insecticide resistance problem and lead to ineffective vector control and resistance management.

We investigated insecticide use practices and the profile of insecticide susceptibility of *Aedes aegypti* mosquitoes occurring at selected hotel compounds on Zanzibar Island. At least 100 adults *Ae. aegypti* females from larvae collected at four hotel compounds were exposed to papers impregnated with discriminant concentrations of DDT (4%), permethrin (0.75%), deltamethrin (0.05%), propoxur (0.1%) and bendiocarb (0.1%) to determine their susceptibility profile.

Allele-specific qPCR and sequencing analysis were applied to determine the possible association between observed resistance and presence of single nucleotide polymorphisms (SNPs) in the voltage-gated sodium channel gene (VGSC) linked to DDT/pyrethroid cross-resistance. Additionally, we explored the possible involvement of Glutathione-S-Transferase gene (*GSTe2*) mutations for the observed resistance profile. In vivo resistance bioassay indicated that, *Ae. aegypti* at studied sites were highly resistant to DDT, mortality rate ranged from 26.3% to 55.3% and moderately resistant to deltamethrin with a mortality rate between 79% to and 100%. However, genotyping of *kdr* mutations affecting the voltage-gated sodium channel only showed a low frequency of the V1016G mutation (n=5; 0.97%). Moreover, for *GSTe2*, seven non-synonymous SNPs were detected (L111S, C115F, P117S, E132A, I150V, E178A and A198E) across two distinct haplotypes, but none of these were significantly associated with the observed resistance to DDT. Our findings suggest that cross-resistance to DDT/deltamethrin at hotel compounds in Zanzibar is not primarily mediated by mutations in VGSC. Moreover, the role of identified *GSTe2* mutations in the resistance against DDT remains inconclusive. We encourage further studies to investigate the role of other potential insecticide resistance markers.

## 4.1. Background

The emergence and spread of insecticides resistances has threaten the effectiveness of vector control interventions and increasing the risk of re-emerging arboviral mosquito-borne arboviral diseases epidemics worldwide, despite substantial investments made to reduce transmission (WHO, 2017a). *Aedes* mosquito species, *Ae. aegypti* and *Ae. albopictus*, are the most widely distributed vectors of the five most important arboviruses associated with explosive epidemics and severe infections, i.e. dengue virus (DENV), yellow fever virus (YFV), chikungunya virus (CHIKV), Zika virus (ZIKV) (Paixão et al., 2018; Powell, 2016) and Rift Valley fever virus (RVF) (Tantely et al., 2015). Dengue fever is the most common and fast-spreading arboviral diseases. An estimated 105 million dengue infections occur each year worldwide including 51 million febrile disease cases (Cattarino et al., 2020). Remarkably, the mainland of Tanzania has experienced several DENV outbreaks within the past decade. The most recent outbreak in 2019 resulted in 6,670 confirmed cases and 13 deaths (WHO, 2019a). Moreover, evidence for possible active transmission of DENV in Zanzibar archipelago has increased (Ali et al., 2020; Gautret et al., 2010; Shauri et al., 2021). This highlights the need for preparedness amid an increasing risk of *Aedes*-borne arboviral disease epidemics in Tanzania, both on the mainland and Zanzibar Islands (Mboera et al., 2016; Saleh et al., 2020b).

General efforts to control mosquito-borne diseases (MBDs) rely heavily on chemical insecticides, mainly delivered in the form of residual spraying and long-lasting insecticide treated nets (Rozendaal, 1997; van Emden & Service, 2004). However, large scale and intensive use of chemical insecticides, both for vector and agricultural pest control, have facilitated the emergence and expansion of resistance in vector populations against virtually all classes of insecticides registered for use in public health (Hemingway et al., 2016; Mota-Sanchez & Wise, 2019). This phenomenon has threatened the gains achieved and the long-term prospect for global elimination of important MBDs, such as malaria and dengue (Hemingway et al., 2016). Moreover, the higher costs associated with the development of novel, safe and efficient chemical insecticides means that existing insecticides will continue to play a pivotal role in the fight against MBDs (van Emden & Service, 2004). Therefore, permanent monitoring of insecticide susceptibility profiles of local vector populations is crucial to detect the emergence of resistance before resistant allele frequencies reach a significant level. This will support the implementation of informed and cost-saving resistance management strategies aimed at reversing resistance or maintaining susceptibility of vector species to available

insecticides. In contrast to malaria vectors (Haji et al., 2013; Haji et al., 2015; Jones et al., 2013), the insecticide resistance profiles of *Aedes* mosquito species occurring in sub-Saharan Africa, including Zanzibar, remains poorly documented. In addition, routine monitoring of insecticide resistance usually targets mosquitoes in and around human settlements. This may lead to the adoption of ineffective resistance management strategies that do not cover residual foci of resistant vector populations. In a recent study, Kampango et al. (2021b) showed that hotels on Zanzibar Island sustain abundant populations of vector mosquito species such as *Ae. aegypti* and *Ae. bromeliae*. Pyrethroids are a widely used class of insecticides, representing more than 30% of the global insecticide market (Zlotikin, 2001). They are the only class of insecticides approved for impregnation of bed nets (WHO, 2006) and are preferred for spraying due to their fast action, relatively low toxicity to humans and short-lived persistence in the environment (van Emden & Service, 2004). In contrast, the use of DDT is restricted to indoor spraying and only recommended under particular circumstances (Rehwagen, 2006). Both pyrethroids and DDT target the transmembrane voltage-gated sodium channels (VGSC) of excitable cells leading to knockdown and death of insects on direct contact (Zlotikin, 2001). Several Single Nucleotide Polymorphisms (SNPs) in the gene coding for VGSC have been linked to pyrethroid and DDT cross resistance in different vector populations of global health concern (Du et al., 2016; Ndiath, 2019). Around thirteen non-synonymous SNPs in the VGSC gene, also known as knockdown resistance (*kdr*) mutations, have been discovered in pyrethroid resistant *Ae. aegypti*. However, only four of them (V410L, S989P, V1016G/I and F1534C) have been frequently associated with DDT/pyrethroid cross-resistance (Moyes et al., 2017; Zuharah & Sufian, 2021). In Africa, the SNPs resulting in F1534C and V1016I mutations have been frequently reported (Kawada et al., 2016; Sombie et al., 2019; Yougang et al., 2020), whereas the V1016G mutation was only recently reported in West Africa (Djiappi-Tchamen et al., 2021). DDT and pyrethroid cross resistance in *Ae. aegypti* have also been linked to metabolic resistance, manifested by increased over-expression of genes coding for detoxification enzymes that metabolize or sequester an insecticide before it reaches the target site (Moyes et al., 2017). This includes several genes of the cytochrome monooxygenase P450 family as well as glutathione-S-transferases (GSTs) (Moyes et al., 2017). Widespread occurrence of cross-resistance mutations can dramatically prevent or delay the implementation of control measures with devastating consequences, not least in epidemic situations. Therefore, thorough knowledge of resistance profiles, and underpinning molecular mechanisms can reduce the frequency of ineffective and wasted insecticide applications and reduce the likelihood of adverse effects on humans and the environment.

The main objective was to outline existing mosquito control practices, determine the status of insecticide susceptibility and molecular mechanisms underpinning DDT/pyrethroids resistance in *Ae. aegypti* population at hotel compounds.

## **4.2. Material and methods**

### **4.2.1. Description of study sites**

Mosquito surveys were carried out from October to November 2019 in four selected hotels located in the Southeast coastal region of Zanzibar Island. Briefly, hotels were selected according to compound size (total residential and non-residential area not less than one hectare), accessibility during low and high tourism seasons, willingness to share data and willingness to accept publication of findings. Consent was obtained from hotel management to conduct the study on their properties. The study sites, extensively detailed in Chapters 2 and Chapter 3, were located at least two kilometres apart. The rainfall regime in Zanzibar archipelago is divided into two main rainy seasons; the long rainy season usually starts from mid-March to June, and the short rainy season from November to December. Average monthly precipitation ranges from 30 mm in July to 320 mm in December and accumulated annual rainfall can reach 1600 mm. *Ae. aegypti* is the most dominant mosquito species found at these hotel compounds and breeds mostly in discarded plastic containers, used tires and tree holes (Chapter 2 and Chapter 3).

### **4.2.2. Mosquito control and insecticide use practices at hotels**

Existing mosquito control practices and insecticide use at hotels were investigated by means of questionnaire surveys administered to hotel staff managers. The questionnaire included questions such as, type of insecticide and regularity of spraying, responsible operators, area covered, costs of interventions, etc (appendix table. A4.1).

### **4.2.3. Mosquito sampling strategy**

#### **4.2.3.1. Collection of mosquito larvae**

To increase the likelihood of sampling enough specimens for bioassays, mosquito sampling was done during the short rainy season, that is, from October to November 2019. At each hotel, immature mosquitoes were surveyed for six to seven days. Details of the adopted sampling strategy have been previously described (Kampango et al., 2021a; Kampango et al., 2021c)

(Chapter 2, and Chapter 3). For relatively small containers [water volume less than 1 litre up to 5 litres, e.g., plastic bottles and beer or soda cans (metal containers)], all larvae and pupae were sampled using pipettes. For large containers (water volume >5 litres, e.g., buckets, jerry cans and ceramic pottery) with relatively few specimens, all larvae and pupae were sampled using dippers, sweep nets or bowls. For the largest containers (water volume > 20 litres, e.g., water tanks, wells, and septic tanks), specimens were collected by 10 - 15 random dips/sweeps. If a container was too deep (e.g., wells) samples were collected using small, suspended buckets (approx. 5 litres), filling ten buckets from different sites of the water container.

#### **4.2.4. Sample processing and identification**

Collected mosquito larvae and pupae were maintained at insectary environmental conditions of  $27 \pm 2^\circ\text{C}$  and  $75 \pm 10\%$  relative humidity until they emerged as adults (WHO, 2016). Larvae were fed ground adult cat food biscuits and kept at 12h light: dark regime. Live adult *Ae. aegypti* were separated from other mosquito species using distinctive morphological features according to taxonomic keys proposed by Huang (2004).

#### **4.2.5. Insecticide resistance phenotypes**

Insecticide susceptibility bioassays were performed according to WHO tube bioassay guidelines (WHO, 2016). Three to five day-old mosquitoes were grouped in batches of 20 to 25 specimens and transferred into holding tubes. Mosquitoes were sugar-fed to reduce the likelihood of death from starvation by placing a cotton wool soaked in a 10% sucrose solution on top of the holding tubes. The holding tubes were maintained inside the testing room for one hour to adjust to these conditions before testing. Female *Ae. aegypti* were exposed to a diagnostic dose of organochlorine DDT (4%), pyrethroids permethrin (0.75%) and deltamethrin (0.05%) and carbamates propoxur (0.1%) and bendiocarb (0.1%). There is currently no consensus on insecticide diagnostic doses for *Ae. aegypti*. Therefore, we used diagnostic doses accepted for malaria vectors, as indicated by WHO insecticide bioassay guidelines (WHO, 2016). The assessed insecticides were selected to represent insecticide classes in use at the hotels during the survey as well as those generally used for public health, mainly deltamethrin and permethrin. DDT was intensively used for malaria control in Zanzibar in the past but was discontinued in 1988 due to the evolution of resistance in *Anopheles* populations (Prapanthadara et al., 2009; Schwartz et al., 1997). However, it was decided to test DDT because the insecticide is still recommended by the WHO for vector control when

resistance to other insecticides is high (Rehwagen, 2006). At least 100 female mosquitoes, divided into replicates of 20-25 specimens, were exposed to each type of insecticide per site. Simultaneously, 50 mosquitoes (25 per cylinder) exposed to papers impregnated with silicone oil acted as negative controls. To characterise knockdown dynamics and estimate the average time to knockdown, the knockdown rate was recorded every 10 minutes over an exposure period of one hour, after which mosquitoes were transferred into recovery cylinders and provided with cotton wool soaked in a 10% sucrose solution. The final mortality rate was estimated 24 hours after the end of exposure, and adjusted with Abbott's correction formula when the mortality in the controls was between 5% and 20% (WHO, 2016). When the mortality rate in the controls was more than 20%, the bioassay was repeated. Mosquitoes were considered susceptible to an insecticide if the final mortality rate was  $\geq 98\%$ , possibly resistant when the mortality rate was between 90% and 97%, and resistant if mortality was  $\leq 90\%$  (WHO, 2016). All specimens exposed to DDT and pyrethroids (permethrin and deltamethrin) that were alive, and a random subsample of twenty specimens from all four hotels that were dead 24 hours after exposure were stored in silica gel at  $-20^{\circ}\text{C}$  for further molecular analysis.

#### **4.2.6. DNA extraction for downstream *Aedes* resistance genes analysis**

For DNA extraction, whole mosquitoes were submerged into liquid nitrogen and ground with a pestle. The mosquito DNA was then extracted using the E-Z 96 Tissue DNA kit (Omega Bio-tek, Norcross, United States) with an adjusted initial step for arthropods as advised by the manufacturer, as follows. Initially, 180  $\mu\text{L}$  of TL buffer and 20  $\mu\text{L}$  of proteinase K were added and then vortexed. The samples were incubated at  $56^{\circ}\text{C}$  overnight until the mosquito was digested (exoskeleton remains excluded). Thereafter, 200  $\mu\text{L}$  of BL buffer was added, and vortexed thoroughly. Finally, 200  $\mu\text{L}$  of 96% ethanol was added, and once again vortexed. After these steps, the rest of the standard extraction protocol was used according to extraction kit manufacturer instructions.

#### **4.2.7. Genotyping of polymorphisms in the VGSC gene**

A subsample of 514 *Ae. aegypti* mosquitoes, comprising specimens found alive ( $n = 333$ ) after being exposed to DDT ( $n=296$ ) or deltamethrin ( $n=37$ ), and dead ( $n = 181$ ) after being exposed to DDT ( $n=96$ ), deltamethrin ( $n=51$ ) or permethrin ( $n=34$ , full susceptibility), were initially analysed for the presence of *kdr* mutation gene by allele-specific qPCR (AS-qPCR). For the genotyping assay for polymorphisms V1016G/I, F1534C, and V410L, mosquito extracted

DNA was investigated by AS-qPCR. Published primer mixes were used consisting of a common reverse primer and two specific primers targeting each polymorphic site (Martins et al., 2009; Stenhouse et al., 2013; Villanueva-Segura et al., 2020). The specificity of the primers was attained for the 3'-end. Additionally, a GC-tail of different length was added to the 5'-end of either the wild type or mutant primer, making them distinguishable (Table A4.2). Real-time PCR reactions were carried out using PerfeCTa FastMix SYBR green (QuantaBio, Beverly, United States). The final reaction had a total volume of 20  $\mu$ L consisting of 10  $\mu$ L SYBR green, 2  $\mu$ L primer mix of 0.2  $\mu$ M (except for the V1016G mutant primer with a concentration of 0.3  $\mu$ M) of each common reverse, forward mutant primer, and a forward wild-type primer, 6  $\mu$ L dH<sub>2</sub>O, and 2  $\mu$ L DNA template. Cycling conditions for SNP F1534C were based on Linss et al. (2014), but with a minor change in elongation temperature (from 60°C to 72°C), resulting in the following programme: first denaturing step of 95°C for 10 min, then denaturing 95°C for 15 seconds, annealing time of 54°C for 15 seconds, and elongation time of 72°C for 30 seconds for 40 cycles (Linss et al., 2014). Cycling conditions for the SNP causing the V1016I change consisted of a denaturing step of 95°C for 10 min, then denaturing 94°C for 30 seconds, annealing time of 62°C for 1 min, and elongation time of 72°C for 45 seconds for 40 cycles (Martins et al., 2009). Cycling conditions for V1016G consisted of a first denaturing step of 94°C for 10 min, then denaturing 94°C for 30 seconds, annealing time of 55°C for 30 sec, and elongation time of 72°C for 30 seconds for 40 cycles (Stenhouse et al., 2013). Cycling conditions for V410L consisted of a first denaturing step of 95°C for 10 min, then denaturing 95°C for 1 min, annealing time of 56°C for 20 sec, and elongation time of 72°C for 20 seconds for 40 cycles (Villanueva-Segura et al., 2020).

#### **4.2.8. Sequencing of domains in the VGSC gene**

Genetic diversity in partial sections of segment S6 in domains I-IV of the VGSC gene were investigated by Sanger sequencing. A total subset of 85 mosquitoes was used to partially sequence segment 6 of domain I-IV of the VGSC to verify the qPCR results and to explore the VGSC gene for other known (S989P, A1007G, I1011M/V, and T1520I) and possibly unknown SNPs. The subset of mosquitoes included 20 sequenced samples in domain I (10 dead, 10 alive), 85 sequenced samples in domain II (25 dead, 60 alive), 28 sequenced samples in domain III (12 dead, 16 alive), and 19 sequenced in domain IV (10 dead, 9 alive). The sections were amplified by conventional PCR, in a reaction volume of 20  $\mu$ L consisting of 11  $\mu$ L dH<sub>2</sub>O, 1  $\mu$ L of each forward and reverse primer with a concentration of 0.20  $\mu$ M, 5  $\mu$ L master mix, and

2 µL of DNA. Each domain was amplified with domain-specific primers (Table A4.3). Briefly, for domain I: initial denaturation of 95°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 1 min with a final elongation of 5 min. For domains II, III, and IV: initial denaturation at 95°C for 10 min, followed by denaturation at 95°C for 30 sec, annealing at 55°C for 1 min and elongation at 72°C for 1 min repeated for 40 cycles followed by a final elongation for 1 min and 30 seconds. Amplicons were verified by electrophoresis (1% agarose gel) and purified using MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, United States) (Table A4.3 for further details on Amplicon sizes). Concentrations in ng/µL of purified amplicons were measured three times for better precision on the NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, United States), adjusted prior to commercial sequencing (Eurofins, Luxembourg). All sequences were compared to reference sequences in Genbank using the MEGA-BLAST algorithm (Morgulis et al., 2008). Consensus sequences were constructed using BioEdit 7.2 (Hall, 1999) with a minimum of 20 bp overlap and 85% similarity. All sequences were then aligned by the MUSCLE algorithm (Edgar, 2004) using the Molecular Evolutionary Genetics Analysis version 10 (MEGA X) (Kumar et al., 2018b).

#### 4.2.9. DNA sequencing of the *Ae. aegypti* *GSTe2* gene

For amplification and sequencing of the *GSTe2* gene, specific primers were constructed according to the reference gene LOC110676856 in the *Ae. aegypti* AaegL5.0 assembly ([https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF\\_002204515.2](https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_002204515.2)). Two primers were constructed to amplify the whole gene sequence and an additional four primers were constructed for Sanger sequencing of the whole gene (Figure A4.2 and Table A4.3). The PCR reaction was carried out with a total volume of 50 µL consisting of 2 µL of each amplification primer (0.20 µM) (Table A4.2), 17 µL of TEMPase Hot Start DNA Polymerase (VWR, Radnor, United States), 24 µL dH<sub>2</sub>O, and 5µL of DNA. The amplification included initial denaturation at 94°C for 15 minutes, cycling consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, elongation at 72°C for 2 min for 35 cycles followed by a final elongation at 72°C for 10 min. Amplicons were verified by electrophoresis (1% agarose gel) and purified using MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, United States). The concentration of purified amplicons was measured three times on a NanoDrop™ 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, United States), and the average concentration used for further calculations. Concentrations in ng/µL of purified amplicons were

measured on the NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, United States), adjusted prior to commercial sequencing (Eurofins, Luxembourg). The procedure for analysis of *GSTe2* gene sequences was similar to analysis of those *VGSC* gene as mentioned above.

#### 4.2.10. Quantification of *GSTe2* gene variants

For detection and quantification of copy number variations (CNV) of the *GSTe2* gene, the concentration of 5  $\mu$ L of dsDNA was measured on a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, United States) using the Qubit dsDNA HS Assay Kit (Invitrogen, Waltham, United States). Since no CNV controls were available, the concentration of the samples was equalized to compare differences in signal from resistant and susceptible mosquito samples. The concentration of the dsDNA was adjusted to be 3 ng/ $\mu$ L. A CNV specific qPCR for *GSTe2* was then performed using specific primers (Table A4.2) at a concentration of 0.32  $\mu$ M. The qPCR assay used a final volume of 20  $\mu$ L consisting of 10  $\mu$ L PerfeCTa FastMix SYBR green (QuantaBio, Beverly, United States), 6  $\mu$ L of dH<sub>2</sub>O, 1  $\mu$ L of each primer, and 2  $\mu$ L of adjusted DNA. The following qPCR conditions were used: an initial denaturation at 95°C for 10 minutes, cycling consisted of 95°C denaturation for 15 seconds, annealing at 55°C for 30 seconds repeated for 40 cycles.

#### 4.3. Statistical analysis

Log-probit regression analyses were applied to predict the probability of *Ae. aegypti* being knocked down by each insecticide during exposure, and to estimate the average time necessary to knockdown 50% (KDT<sub>50</sub>) and 95% (KDT<sub>95</sub>) of *Ae. aegypti* populations when in contact with each type of insecticide. Regression models were fitted using the R software package drc v. 3.0-1 (Ritz & Streibig, 2005). Fisher exact tests were applied to determine whether the difference between the frequencies of insecticide resistance polymorphisms was significant. A simple t-test was applied for CNV to investigate any significant difference in qPCR Ct-values in the *GSTe2* gene between susceptible and resistant *Ae. aegypti* samples.

## 4.4. Results

### 4.4.1. Mosquito control activities and insecticide use practices at hotels

Questionnaire surveys on insecticide use practice administered to hotel managers indicate that all four hotels have implemented mosquito management intervention by mean of chemical control, namely indoor space spraying, fogging (restaurant, lobby, gardens, open space), use of long-lasting insecticidal bed nets (staff quarter) and locally manufactured untreated bed nets (guest rooms) (Table 4.1).

**Table 4.1.** Mosquito control practices, type of insecticide, quantity, and frequency of use at hotel compounds in Zanzibar

| Hotel   | Methods            | Target site | Type of insecticides (Concentration)  | Class                | Frequency |
|---------|--------------------|-------------|---------------------------------------|----------------------|-----------|
| Hotel A | Space spraying     | Indoor      | Propoxur/D-trans-allethrin (250 mg/L) | Carbamate/Pyrethroid | Daily     |
|         | Fumigation/Fogging | Indoor      | Dichlorvos (500mg/L EC)               | Organophosphate      | Monthly   |
|         | Fumigation/Fogging | Outdoor     | Dimethoate (400mg/L EC)               | Organophosphate      | Monthly   |
| Hotel B | Space spraying     | Indoor      | Propoxur/D-trans-allethrin (250 mg/L) | Carbamate/Pyrethroid | Daily     |
|         | Fumigation/Fogging | Indoor      | Dichlorvos (500mg/L EC)               | Organophosphate      | Monthly   |
|         | Fumigation/Fogging | outdoor     | Deltamethrin (250 mg/L EC)            | Pyrethroid           | Fortnight |
| Hotel C | Space spraying     | Indoor      | Propoxur/D-trans-allethrin (250 mg/L) | Carbamate/Pyrethroid | Daily     |
| Hotel D | Space spraying     | Indoor      | Propoxur/D-trans-allethrin (250 mg/L) | Carbamate/Pyrethroid | Monthly   |
|         | Repellent          | Outdoor     | Unknown                               | Unknown              | daily     |

EC – Emulsified concentration

The most common types of insecticides used for indoor space spraying was carbamate propoxur, sometimes mixed with the pyrethroid D-trans-allevethrin, purchased in small tins of 340 ml and 1400 ml. For outdoor fumigation and fogging the most common types of insecticide used were the organophosphate dichlorvos, dimethoate and pirimiphos-methyl (Table 4.1). The pyrethroids mostly used were deltamethrin. Fogging was the mosquito control practice only at Hotel A and Hotel B. Topical use repellents (MedSoft™) were occasionally supplied to guests at Hotel D.

#### 4.4.2. Insecticide susceptibility

A total of 2,561 *Ae. aegypti* females were tested (Hotel A: n = 400 specimens; Hotel B: n = 836; Hotel C: n = 775; Hotel D: n = 550). The results of probit regression analysis are depicted in Figure 4.1 and summarized in Table 4.1. Mosquitoes from Hotel A were tested with only four insecticides due to too few specimens being available. In general, *Ae. aegypti* specimens from all four hotels showed the slowest knockdown rate when exposed to DDT (Figure 4.1. and Table 4.2).

The estimated average knockdown time KDT<sub>50</sub> of DDT exposed mosquitoes varied from 61.1 (95% CI, 56.6 - 65.5) among specimens from Hotel D to a maximum of 152.7 (95% CI, 84.7 - 220.7) minutes for specimens from Hotel B. Mortality rates estimated twenty-four hours post-exposure ranged from 26.3% (Hotel B) to 55.3% (Hotel D) indicating presence of resistance to DDT in *Ae. aegypti* populations from all four hotels (Table 4.1). Results also suggest moderate resistance to deltamethrin, with mortality rates ranging from 79% (Hotel B) to 100% (Hotel D), and possible low levels of resistance to propoxur in the population from Hotel D, where the mortality rate was 96%. Full susceptibility was observed with permethrin and bendiocarb across all study sites (Table 4.1).

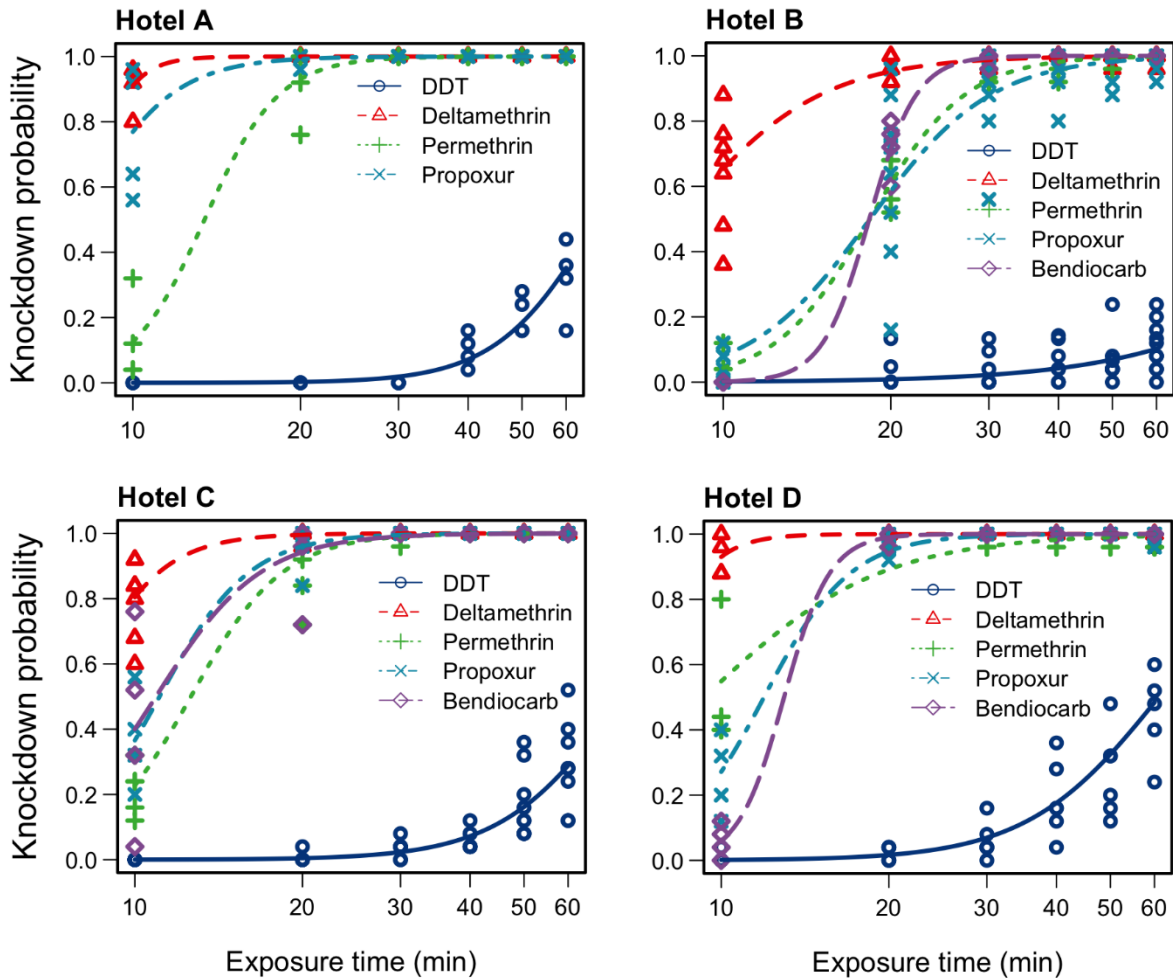


**Figure 4.1.** Knockdown probability of *Aedes aegypti* exposed to discriminant doses of insecticides. Consistent delayed knockdown of *Ae. aegypti* exposed to DDT is visible at all sites, which indicates resistance.

**Table 4.2.** Estimates of mortality rates and average exposure time necessary to knockdown *Aedes aegypti* exposed to insecticides. Shaded rows indicate resistance or suspected resistance requiring confirmation.

| Hotel   | Insecticide  | Total tested/<br>Replicate No. | KDT <sub>50</sub> (± 95 % CI) | KDT <sub>95</sub> (± 95 % CI) | Number dead | Mortality rate |
|---------|--------------|--------------------------------|-------------------------------|-------------------------------|-------------|----------------|
| Hotel A | DDT          | 100/4                          | 68.1 (61 - 75.1)              | 125.3 (90.6 - 160.1)          | 39          | 39%            |
|         | Deltamethrin | 100/4                          | 8.1 (1.6 - 14.6)              | 10.6 (8.2 - 13)               | 99          | 99%            |
|         | Permethrin   | 100/4                          | 7.95 (6.66 - 9.24)            | 13.94 (11.51 - 16.38)         | 100         | 100%           |
|         | Propoxur     | 100/4                          | 7.9 (6.7 - 9.2)               | 13.9 (11.5 - 16.4)            | 100         | 100%           |
| Hotel B | DDT          | 236/10                         | 152.7 (84.7 - 220.7)          | 541 (53.7 - 1028.3)           | 62          | 26%            |
|         | Deltamethrin | 200/8                          | 8.3 (7.4 - 9.2)               | 19.7 (17.4 - 22.1)            | 158         | 79%            |
|         | Permethrin   | 200/8                          | 18.4 (17.4 - 19.3)            | 38.3 (35.2 - 41.3)            | 196         | 98%            |
|         | Propoxur     | 100/4                          | 18.3 (17.1 - 19.6)            | 32.7 (29.4 - 36)              | 99          | 99%            |
|         | Bendiocarb   | 100/4                          | 18.3 (17.3 - 19.3)            | 24.3 (22.2 - 26.5)            | 98          | 98%            |
| Hotel C | DDT          | 225/10                         | 74.9 (67.4 - 82.3)            | 154.3 (115.2 - 193.3)         | 96          | 43%            |
|         | Deltamethrin | 200/8                          | 7.9 (6.8 - 8.9)               | 13 (11.3 - 14.6)              | 180         | 90%            |
|         | Permethrin   | 150/6                          | 12.6 (11.9 - 13.4)            | 22 (19.8 - 24.3)              | 150         | 100%           |
|         | Propoxur     | 100/4                          | 10.9 (10.1 - 11.8)            | 20.6 (17.6 - 23.6)            | 100         | 100%           |
|         | Bendiocarb   | 100/4                          | 11.1 (10.3 - 11.8)            | 19.1 (16.4 - 21.9)            | 100         | 100%           |
| Hotel D | DDT          | 150/6                          | 61.1 (56.6 - 65.5)            | 136.8 (106.8 - 166.9)         | 83          | 55%            |
|         | Deltamethrin | 100/4                          | 7.4 (2.4 - 12.4)              | 10.4 (9.1 - 11.7)             | 100         | 100%           |

|            |       |                    |                    |     |      |
|------------|-------|--------------------|--------------------|-----|------|
| Permethrin | 100/4 | 11.9 (11.1 - 12.8) | 20.2 (17.5 - 22.9) | 100 | 100% |
| Propoxur   | 100/4 | 9.3 (7.9 - 10.7)   | 27.1 (22.4 - 31.8) | 96  | 96%  |
| Bendiocarb | 100/4 | 13 (11.9 - 14.1)   | 17.1 (14.7 - 19.6) | 100 | 100% |

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#### 4.4.3. Frequency of SNPs in the VGSC gene

Molecular analysis targeting mutations in the VGSC gene identified only five mosquitoes that were heterozygous at V1016G. These five mosquitoes were all DDT resistant as determined by the insecticide susceptibility bioassay, and all were from the same site (Hotel B). Results of nucleotide sequences of partially sequenced segment 6 of domain I-IV of the VGSC were identical to the results obtained by qPCR. No previously described or new SNPs were detected in the VGSC gene (Figure 4.2).

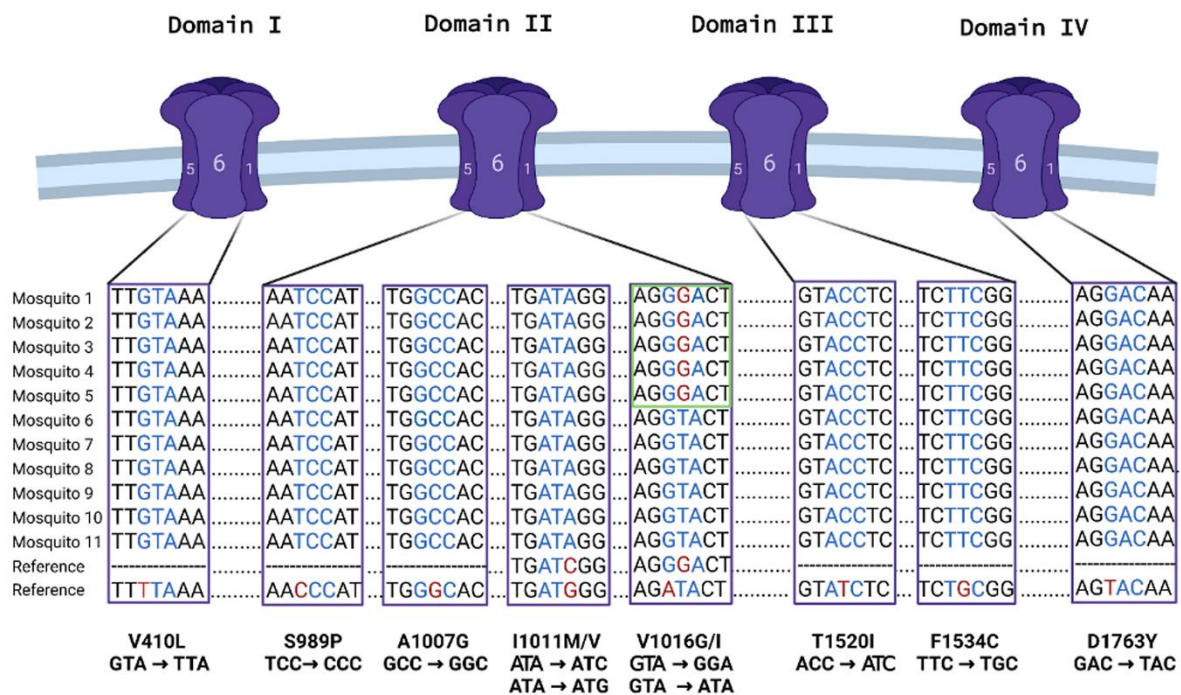


**Figure 4.2.** Alignment of eleven representative nucleotide sequences from DDT and deltamethrin resistant *Ae. aegypti* samples. The reference sequences are constructed to contain mutant codons and the sequence obtained from each of the VGSC structure domains (I - IV) are also shown. The green box highlights the five V1016G mutants detected through allele-specific qPCR in segment six (S6) of domain II that were verified by Sanger sequencing.

#### 4.4.4. Polymorphisms and CNV in the GSTe2 gene

A total of 57 mosquitoes were PCR positive for presence of the GSTe2 gene. Due to resource limitations, 47 confirmed positive samples were chosen for purification and sequencing, and 21 mosquitoes were successfully sequenced in the whole GSTe2 gene (Five susceptible and 16

resistant). Seven non-synonymous SNPs resulting in the following changes: L111S, C115F, P117S, E132A, I150V, E178A and A198E. These substitutions were consistently distributed across two distinct haplotypes. Thirteen of the 21 sequenced haplotypes belonged to haplotype 1 (4 susceptible and 7 resistant) and 8 belonged to haplotype 2 (1 susceptible and 7 resistant) (Figure A4.2). Haplotype 2 showed low similarity (~90% homology) to available sequences in Genbank, whereas haplotype 1 showed ~96% homology. However, no statistically significant association between haplotype variant and insecticide resistance was found ( $p = 0.65$ ). Regarding CNV, no statistically significant difference was found between the qPCR Ct values measured for 14 DDT susceptible (mean Ct value = 20.70 (range 19.08 -22.34) and 15 DDT resistant (mean Ct value = 20.56 (range 19.43-21.76) *Ae. aegypti* samples ( $p = 0.61$ ).

#### 4.5. Discussion

Accurate knowledge of mosquito susceptibility to available insecticidal control measures and the underlying environmental and biological mechanisms conferring resistance is crucial for selection of appropriate vector control strategies and implementation of cost-saving resistance management practices. To the best of our knowledge, this is the first attempt to investigate the status of insecticide resistance and possible association to molecular alterations in *Ae. aegypti* populations of the Zanzibar archipelago.

*In vivo* susceptibility bioassays indicated that, *Ae. aegypti* populations at four hotel sites were highly resistant to DDT, moderately resistant to deltamethrin and possibly resistant to propoxur, while fully susceptible to permethrin and bendiocarb. The insecticide susceptibility status of arboviral vectors, not least *Ae. aegypti*, remains poorly investigated in Tanzania, as indeed in most of the East African region (<https://aedes.irmapper.com>), with the few published reports limited to phenotypic characterisation of resistance. This includes a recent survey of *Ae. aegypti* in the Ifakara area of south-eastern Tanzania, showing full susceptibility to deltamethrin, permethrin, bendiocarb and pirimiphos-methyl (Kahamba et al., 2020) and a study from Dar es Salaam, in which *Ae. aegypti* displayed resistance to deltamethrin and lambda-cyhalothrin, but varying susceptibility to permethrin (2017).

The lowest mortality rate of mosquitoes exposed to DDT was observed at Hotel B, whereas mortality rates from the remaining three hotels were statistically comparable. DDT was for many years an integral part of antimalarial house-spraying campaigns in Zanzibar. However, it was fully withdrawn in 1988 due to high levels of DDT resistance in *An. gambiae s.l.*, the

dominant malaria vector complex in the region (Schwartz et al., 1997). An exploratory survey of mosquito control practices at hotels, carried out prior to this study, did not find any evidence of recent use of DDT or analogue insecticides at the study hotels (Table 4.1). Hotels had implemented periodic outdoor spraying with an emulsified concentrate of the organophosphate dichlorvos and the pyrethroids deltamethrin, D-trans-alallethrin (Table 4.1). Space spraying with deltamethrin was also performed periodically at Hotel B, which may somehow explain the high level of deltamethrin resistance observed at Hotel B. The four hotels performed aerosol (indoor) spraying using locally available commercial canned mixtures of the pyrethroids imiprothrin and cypermethrin, or with a formulation of the carbamate, propoxur. All of the above-mentioned insecticides are currently registered for use in Tanzania (Tropical Pesticides Research Institute (TPRI), 2020). With 40 years having elapsed since the last widespread use of DDT to control *Anopheles* mosquitoes it is notable that *Ae. aegypti* in Zanzibar are resistant to this insecticide. However, mosquitoes can retain resistance to DDT over long periods. For instance, field studies in India on the malaria vectors *An. stephensi* and *An. culicifacies* showed that these species had retained resistance to DDT thirty years after the use of this insecticide was officially terminated (Raghavendra et al., 2010). Contrarily, other published evidence shows that slow reversion toward DDT susceptibility can occur in wild populations of *An. stephensi* and *Ae. aegypti* when the DDT pressure is removed (Abedi & Brown, 1960; Bang et al., 1971). As such, we propose that the past intense use of dieldrin (1958 - 1961), and thereafter DDT (until 1988), as part of malaria eradication campaigns in Zanzibar (Lines & Nassor, 1991; Schwartz et al., 1997), may have led to unintended selection of DDT resistance in local *Ae. aegypti* populations. Unfortunately, studied hotels did not keep historical records of insecticide use in their premises which has prevented us from making direct connection between observed phenotypic resistance to DDT and any past use of DDT at studied hotel compounds.

Allele-specific qPCR-based assays detected the presence of heterozygous V1016G mutant alleles in the VGSC gene of five specimens of *Ae. aegypti* (found at Hotel B). No other investigated point mutations commonly found in *Ae. aegypti* were detected. This finding contrasts with the outcome of our phenotypic resistance bioassay, which indicated higher resistance to DDT and moderate resistance to deltamethrin. The geographical occurrence of the V1016G mutation is expanding in the Middle East (Al Nazawi et al., 2017), southeast Asia (Kawada et al., 2009; Kawada et al., 2014; Li et al., 2015; Srisawat et al., 2010; Stenhouse et al., 2013) and Latin America (Murcia et al., 2019). Notably, it was recently reported in Cameroon, West Africa (Djiappi-Tchamen et al., 2021). The origin of V1016G/I mutations in

African populations of *Ae. aegypti* remains poorly understood. Fan et al. (2020) proposed that the variant may have arisen from genetic recombination in the evolution of *kdr* alleles or by introduction from another continent. We did not observe a clear association between the presence of the V1016G alleles and reduced susceptibility to DDT and deltamethrin in *Ae. aegypti* from Zanzibar. The lower frequency of V1016G alleles suggests recent introduction or occurrence of this type of *kdr* mutation in *Ae. aegypti* populations at the studied sites. It may also suggest reduced fitness of V1016G alleles and might be unfavourable to maintain. As noted earlier, there is intensive pesticide use at the studied hotels, which may have applied selection pressure for resistance in the *Ae. aegypti* populations. Therefore, further investigations are encouraged to understand factors driving the emergence and propagation of *kdr* mutations in order to prevent their successful establishment and expansion throughout the Zanzibar archipelago.

Several genes of the family cytochrome P450 and glutathione-S-transferases (GSTs) have been associated with pyrethroid and DDT resistance in *Ae. aegypti* populations (Sene et al., 2021; Smith et al., 2019; Sombie et al., 2019). However, in this study, sequencing analyses indicated no significant correlation between *Ae. aegypti* resistance to DDT and presence of GSTe2 SNPs observed (L111S, C115F, P117S, E132A, I150V, E178A, and A198E). In Senegal, Sene et al. (2021) also reported higher phenotypic resistance to DDT and permethrin in *Ae. aegypti* but like this study, no *kdr* mutations were detected. The authors found overexpression of genes of the P450 family namely *CYP6BB2*, *CYP9J26* and *CYP9J32*, associated with resistance to pyrethroids and DDT, and high overexpression of the *GSTD4* glutathione-S-transferase gene, associated with resistance to DDT. This study did not investigate the potential role of P450 family genes since, in general, *Ae. aegypti* showed susceptibility to the two types of pyrethroids tested, excepting at Hotel B where resistance to deltamethrin was detected. Using lab-based resistance selection experiments, Smith et al. (2019) showed that cytochrome P450s genes can also mediate cross resistance to pyrethroids and organophosphates in *Ae. aegypti* either in the presence or absence of *kdr* mutations, underscoring the complexity of molecular mechanisms of insecticide resistance in *Ae. aegypti* populations. No significant association was detected between *GSTe2* polymorphisms and phenotypic resistance to DDT, suggesting that a potentially different resistance mechanism may be involved. There are many other poorly investigated resistance mechanisms to pyrethroids and DDT, such as the recently described cuticular-based resistance to pyrethroids and DDT in *Ae. aegypti* from Cameroon (Borel et al., 2021b). Cuticle-based resistance mechanisms have been systematically neglected. However,

recent evidence showing its role in resistance against pyrethroids, and DDT serves as a sharp reminder that further in-depth investigations on the process underlying cuticular-based resistance may significantly impact the design of resistance management tools. Finally, previous studies with *An. arabiensis* and *An. gambiae* s.s. from Zanzibar reported an eight-fold higher increase of DDT-dehydrochlorinase activity of glutathione S-transferases in resistant populations compared to susceptible ones (Prapanthadara et al., 1995). As such, we encourage further investigations into the probable involvement of other, less describe or rare mechanisms that might underpin pyrethroid/DDT resistance in *Ae. aegypti* and similar vectors of public health concern.

#### 4.6 Concluding remarks

This study shows that resistance to pyrethroids and DDT is present in *Ae. aegypti* of Zanzibar. It also shows, for the first time, the presence of the *kdr* mutation V1016G in the region. However, any association between the V1016G mutation and resistance to pyrethroids and DDT in *Ae. aegypti* populations remain inconclusive due to low detection frequency of the resistant allele. Emergence of V1016G *kdr* mutations in Zanzibar should be of great concern given the intense use of pyrethroids in and around hotels. Successful expansion of V1016G throughout the archipelago would compromise the effectiveness of vector control by both type 1 and type 2 pyrethroids with devastating consequences in the event of an *Aedes*-borne disease epidemic. Therefore, efforts should be made to reduce selection pressure by replacing the use of chemical control by environmental mosquito management practices. Our findings also highlight the need for continued and comprehensive investigations on other potential mechanisms for pyrethroid resistance in local *Ae. aegypti* populations. Moreover, the risk of large-scale use of insecticides for mosquito control at private facilities, outside direct government control, should be thoroughly investigated.

#### 4.7. Acknowledgements

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## CHAPTER 5

### **A framework for evaluating the entomological impact of larval source reduction on mosquito vectors at hotel compounds in Zanzibar**

Ayubo Kampango, Peter Furu, Flemming Konradsen, Fatma Saleh, Michael Alifrangis, Karin L. Schiøler and Christopher W. Weldon

This chapter addresses objective seven of the thesis, and it will be adapted for planned publication as a protocol.

## Abstract

There is a growing awareness of the association between tourism activity and risks of emerging mosquito-borne diseases (MBDs) worldwide. In previous studies we showed that hotels in Zanzibar may play an important role in maintaining residual foci of mosquito vector populations of public health relevance. These results showed that larval habitat removal may have a significant negative impact on vector community structure. However, a thorough analysis of the response of local vector species to potential larval source management (LSM) strategies must be evaluated prior to implementation of a large-scale area-wide vector control campaign. As such, a framework for hotel-based entomological assessment of the efficacy of larval source reduction (LSR) against container-breeding mosquito vector populations at hotel settings in Zanzibar is proposed. This framework is set to determine the efficacy of LSR in a randomized partial cross-over experimental design with four hotel compounds representing the unit of randomization for allocation of treatments. Proposed treatments are active removal of disposed containers and installation of water refilling machines to replace the use of single-use plastic water bottles, which are the most important source of mosquitoes identified in the studied hotels. We propose that the treatment should at first be allocated to two hotel compounds during the dry season (January – March), when mosquito abundance is predictably lower. The possible impact of interventions would then be evaluated throughout subsequent seasons, namely rainy (April – June and November – December) and dry (July – August and January - March) over six to seven months. If an appreciable effect of interventions on mosquito population is observed during the trial period, treatment could be extended to the two previously untreated hotels to determine whether any potential reduction of mosquito density is reproducible. The investigation is expected to be conducted over eighteen months. A rigorous evaluation of the proposed vector-control measures will provide support for developing an evidence-based, ecologically sound, effective, and sustainable mosquito control at hotel facilities and will also inspire large-scale trials.

## 5.1. Background

Mosquitoes are vectors of several important parasitic and viral diseases that affect human health and halt economic and societal progress in endemic countries (Wilkerson et al., 2021). Malaria, dengue, chikungunya, yellow fever, Zika, Japanese encephalitis and lymphatic filariasis are the most notorious mosquito-borne diseases (MBDs) (WHO, 2020a). They are present across tropical and subtropical countries, with more than half of the world's population currently at risk (WHO, 2020a).

Tanzania is one of four countries that in 2020 accounted for half of global reported malaria deaths (WHO, 2021). The country has also become more prone to mosquito-borne viral disease outbreaks, as evidenced by an unprecedented number of dengue virus outbreaks since 2010 (Chipwaza et al., 2021b; Mwanyika et al., 2021; Ward et al., 2017). The most recent outbreak occurred on the mainland in 2019, resulting in 6670 confirmed cases including 13 deaths (WHO, 2019a). Remarkably, in the Zanzibar archipelago, dengue or related mosquito-borne viral disease outbreaks have not been officially reported since 1823 (Christie, 1872). However, there is a high risk of a devastating *Aedes*-borne disease epidemics (Mweya et al., 2016; Saleh et al., 2020a). Factors such as widespread infestation by vector species (Kampango et al., 2021a; Kampango et al., 2021c; Saleh et al., 2018), extensive connectivity between Zanzibar, mainland Tanzania and other endemic regions (Le Menach et al., 2011; Morgan et al., 2020), rapid demographic expansion (Mwalusepo et al., 2017), as well as poor solid waste management (UNICEF, 2018) may precipitate the emergence of outbreaks in the region. Local studies and clinical reports of visitors returning from Zanzibar strongly suggest that there has been silent circulation of dengue and chikungunya virus in the archipelago (Ali et al., 2020; Gautret et al., 2010; Shauri et al., 2021). This underscores the need to identify and eliminate the triggers of potential *Aedes*-borne disease epidemics in the region.

The impact of tourism on mosquito-borne disease expansion, and outbreak risks is gaining long-overdue global attention (Rossello et al., 2017). Tourism promotes connectivity between different sites, including endemic regions, and thus increases the likelihood of introducing novel pathogens into new environments and exposes immunologically naïve visitors to potentially deadly pathogens at endemic travel destinations. Therefore, it is crucial for popular tourist destinations such as Zanzibar to implement actions that would reduce the risk of epidemics. Reduction of the potential threat of epidemics is vital, given the limited readiness of the Zanzibar healthcare system for management and control of mosquito-borne viral diseases

(Saleh et al., 2021). In previous studies (Chapter 2, Chapter 3, Chapter 4), we have shown that hotels in Zanzibar may provide suitable conditions for sustaining mosquito vectors of global concern, notably *Ae. aegypti*, *Ae. bromeliae*, *Ae. africanus*, *Ae. metallicus*, *Cx quinquefasciatus*, *Er. quinquevittatus*, *Er. chrysogaster* and, to less extent, *An. gambiae* (Kampango et al., 2021a; Kampango et al., 2021c). These vectors utilize discarded water-holding containers and domestic utensils as larval habitats, mainly plastic water bottles, plant pots, cans, and tanks (Kampango et al., 2021a; Kampango et al., 2021c). Occurrence of such vectors aligns with increasing visitor reports of mosquito bites during their stay at hotels in Zanzibar. The fear of being infected by life-threatening mosquito-transmitted pathogens has been pointed out by visitors as one of the main reasons for low interest in return to the region. Some visitors may not even consider Zanzibar in the first place, as the apparent risk of disease exposure governs the choice of tourism destination (Rossello et al., 2017). The tourism industry would be severely affected, in case of epidemic events, by a devastating impact on the economic stability of Zanzibar, as observed in several other insular regions strongly reliant on tourism (Deisting & Rey, 2015; Duman-Scheel et al., 2018; Francis et al., 2021; Yoshikawa et al., 2020). Furthermore, we have also showed that resistance to DDT and pyrethroid insecticides is already established in the major arboviruses vector species *Ae. aegypti*, while carbamate resistance may be emerging (Kampango et al., 2022). These findings strongly suggest that the studied hotels qualify for environmental mosquito management interventions, by means of larval source management (LSM), to control mosquito populations (WHO, 2013).

The management of mosquitoes at hotel compounds has long relied on insecticide-based population suppression measures, notably outdoor space spraying/fogging and use of LLINs (Kampango et al., 2022). However, these interventions only target endophagic and endophilic vector species, while a large fraction of exophagic and exophilic species remain unaffected. Control approaches that selectively target a small fraction of important vector populations may likely lose support if non-targeted vectors or nuisance species remain unaffected (WHO, 2013). Additionally, both residual spraying and bed nets have proven inefficacious against important day-biting, container-exploiting mosquito vectors, such as *Ae. aegypti*, in several contexts (Bowman et al., 2016), possibly due to a remarkable and widespread level of insecticide resistance (Moyes et al., 2017). Therefore, wasteful use of inefficacious insecticides at hotel compounds can exacerbate the resistance selection problem and cause irreparable environmental harms. Thus, novel, effective and environmentally friendly control measures

that ensure the integrity of non-target insect biodiversity, and economic development, is recommended (The World Tourism Organization (UNWTO), 2022).

Several vector control efficacy randomized trials have shown that community-based mosquito larval source reduction (LSR) interventions, coupled with consistent surveillance can result in a meaningful reduction of mosquito vector populations in several endemic settings elsewhere (Andersson et al., 2015; Trewin et al., 2017a; Vanlerberghe et al., 2010), including Tanzania (Castro et al., 2010; Castro et al., 2009; Geissbuhler et al., 2009). Moreover, several vector species share larval habitats (Kampango et al., 2021c). As such, LSR has the potential for controlling both vectors and nuisance mosquito species. Therefore, we argue that a successful control of mosquito populations at studied hotels in Zanzibar would require the implementation of environmental control measures to remove all potential mosquito larval sources and, at the same time, ban the use of discardable plastic water bottles due to their status as the most productive mosquito larval habitats found at hotel compounds (Kampango et al., 2021a; Kampango et al., 2021c). Discarded water bottles eventually provide suitable larval habitats for mosquitoes given the limited solid waste removal practices we observed at the study sites during the undertaking of previous surveys. We encourage the substitution of single use plastic water bottles by onsite water refilling practices and introducing reusable bottle solutions at hotel compounds. In Thailand, the replacement of single use plastic water bottles by water refilling practices at a hotels, resorts and spas complexes has been shown to prevent the production of one million plastic bottles annually, that would otherwise be discarded in the environment (Six Sense, 2021). However, the potential impact of this approach on the populations of container-exploiting mosquitoes has, to the best of our knowledge, never been tested before.

Hotels present an ideal environment to test novel mosquito control interventions given the management set up, resources, staff availability and economic incentives for providing a mosquito-safe sustainable environment. However, prior to implementation of large-scale trials, it is crucial to undertake feasibility assessments to determine whether selected intervention approaches are technically, operationally, and socio-economically practical (Stevens, 1984; WHO, 1982; 2013). Feasibility studies form the basis for estimating the required resources (material and manpower) and the cost of a large-scale trial addressing the eco-geographic and environmental spectrum of mosquito occurrence in hotels in Zanzibar. Furthermore, they allow for estimation of the level of acceptability and compliance to the LSR approaches by

management, staff, and visitors. As such, we propose a tool for evaluating the entomological impact of environmental larval source management on mosquito population occurrence and abundance at hotel compounds in Zanzibar through integration of LSR actions and implementation of onsite water-refilling practices. We anticipate that eliminating potential mosquito larval sources from the environment and cutting the production of disposable plastic water bottles will deprive local mosquito fauna of key larval habitats and reduce or eliminate mosquito populations from the hotel compounds.

## 5.2. Materials and methods

### 5.2.1. Study sites

The study sites are described exhaustively in previous chapters and Kampango et al. (2021c); Kampango et al. (2021a); Kampango et al. (2022). The hotels are in the South-East coastal region of Zanzibar Island, the largest island of Zanzibar archipelago located in the Indian ocean (Figure 5.1). The island is about 85 km long and 39 km wide, and occupies a total surface area of 1,464 km<sup>2</sup> (AfricaGuide, 2019). The topography is mainly dominated by lowland areas, with the highest point at 120 metres above sea level (AfricaGuide, 2019). According to the last population census (2012), an estimated 896,721 people live on the island, corresponding to 1,554 inhabitants/square kilometre. The eastern part of the island is arid and covered in coral rag (rock made of coral) making it attractive and ideal for fishing villages as well as the tourist and recreation industry, representing the third largest economy of the island (Forum, 2019b). *Anopheles arabiensis* is the most dominant malaria vector (Bjorkman et al., 2019; Haji et al., 2013). However, arboviral disease vectors viz., *Ae. aegypti*, *Ae. bromeliae*, *Ae. metallicus* and *Ae. africanus* also occur. (Kampango et al., 2021a; Kampango et al., 2021c; Saleh et al., 2020a). There are indications of dengue and chikungunya transmission in Zanzibar, but the main vector species are yet to be incriminated.

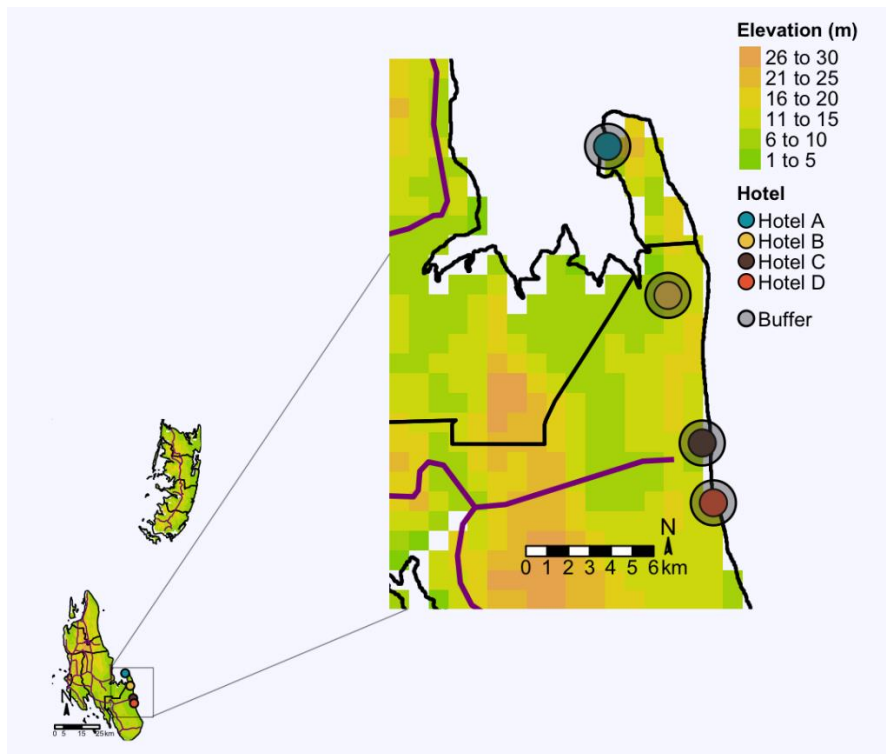


**Figure 5.1.** Location on the east coast of the southern region of Zanzibar (Unguja) Island. Grey circles around each location indicate a 300 metres buffer radius for deployment of interventions. Dark magenta lines depict main roads. Map made by author. Elevations raster grid and administrative borders shapefile obtained from <https://gadm.org>, Zanzibar main roads shapefile obtained from <http://www.naturalearthdata.com>.

### 5.2.2. Recruitment and eligibility

The hotels were selected according to compound size (total residential and non-residential area not less than one hectare), accessibility during lower and higher tourism seasons, willingness to share data and willingness to accept publication of findings.

### 5.2.3. Capacitation development and training

Mosquito larval habitat reduction actions need to be integrated into hotel daily routines. Therefore, designated hotel staff members will receive a series of tailored, hands-on training on the identification of potential mosquito larval habitats and mosquito aquatic stages, prior to and throughout the implementation of control measures. This equips them with the competencies and proper technical tools required to implement control measures, and for

gathering information for monitoring and evaluation of the impact of these measures, such as larval habitat characteristics, occurrence, and abundance of mosquito immature stages, assembling, deployment and maintenance of mosquito traps, and collection of adult mosquitoes, samples preservation, etc). A training syllabus will be designed, potentially in collaboration with the Zanzibar Malaria Elimination Programme (ZAMEP) and be taught through planned workshops.

#### **5.2.4. Description of interventions**

Environmental manipulation (WHO, 1982) interventions will be evaluated. These comprise actions to remove discarded natural and artificial containers, which are sites for mosquito reproduction at hotel compounds. Concurrently, portable water dispensers will be deployed at hotel facilities to replace discardable plastic water bottles. Water dispensers will be set up in most frequented areas at hotel facilities, such as restaurants, lounges, and entry foyers. Wherever justifiable and logistically practical, habitat modification measures, such as drainage, covering or filling of containers (e.g., tree holes), will also be implemented to prevent them from collecting rainfall or any sort of flowing or gardening water that might support mosquito immatures stages.

#### **5.2.5. Study design**

We propose a randomized partial cross-over experimental design to evaluate the impact of proposed interventions (Hutson & Reid, 2004; Wilson et al., 2020). Therefore, the unit of randomization for allocation of interventions is a hotel compound. Two of the four hotels will be chosen to receive the treatments, that is, physical removal of larval habitats and set up of water dispensers, and the remaining two hotels will be the control. The choice of hotels to receive the interventions will be carried out through a computer-based randomization. Hotels will be required to stop implementing outdoor space spraying activities during the trial period to eliminate a possible confounding factor. A potential replacement for space spraying could be deployment of transfluthrin emanators. Transfluthrin is a fast-acting spatial repellent whose efficacy in reducing the risk of human exposure to malaria, lymphatic filariasis, dengue, and other arbovirus vectors has been demonstrated (Mmbando et al., 2018; Morrison et al., 2022; Ogoma et al., 2017). The acquisition of transfluthrin could be commissioned between the project and hotel managements.

### 5.2.6. Intervention allocation

The interventions will be allocated to intervention arms during the dry season (January – March) (Figure 5.2), when mosquito abundance is predictably lower (Kampango et al., 2021a; Kampango et al., 2021c), so that the actual impact on mosquito populations is evaluated throughout subsequent rainy and dry seasons for the entire temporal range of mosquito occurrence, as recommended by WHO (2013). Specifically, treatment will be allocated in two phases; in the first phase it is assigned only to the intervention arm (January – March) and the impact then monitored over six months covering the long rainy season (April – June) and the dry season (July – September). In the second phase, interventions will be extended to the two untreated hotels (control arm) in October (dry season), provided that an appreciable effect is observed during phase 1. This will show whether any potential reduction of mosquito density and occurrence probability observed in the intervention arm can be reproduced and maintained in the control arm. Wherever possible, larval habitat removal control interventions will be expanded to surrounding areas to maintain a buffer zone of 300 metres radius around each treated hotel (Figure 5.1). The choice of 300 metres radius for the buffer zone is based on the current knowledge of maximum flight range of Afrotropical *Aedes* mosquito vectors (Guindo-Coulibaly et al., 2019; Marcantonio et al., 2019; Verdonchot & Besse-Lototskaya, 2014).

| Intervention allocation | Intervention impact evaluation (Follow-up) |              |     |            | Interventions extended to control arm (impact evaluation follow-up) |              |  |            |  |
|-------------------------|--------------------------------------------|--------------|-----|------------|---------------------------------------------------------------------|--------------|--|------------|--|
| Dry season              | Rainy season                               |              |     | Dry season |                                                                     | Rainy season |  | Dry season |  |
| Jan Feb Mar             | Apr May Jun                                | Jul Aug Sept | Oct | Nov Dec    | Jan Feb Mar                                                         |              |  |            |  |
|                         |                                            |              |     |            |                                                                     |              |  |            |  |
|                         |                                            |              |     |            |                                                                     |              |  |            |  |



**Figure 5.2.** Schematic representation of the allocation and timing of interventions to hotels as part of the partial cross-over experimental design.

### 5.2.7. Sample size calculation

Provisional sample size is calculated assuming that the surveys are expected to be carried out for 24 months, and at each hotel mosquito populations data collections should be undertaken for 7 days per month. Therefore, we expect to have at least  $n = 168$  replicate-days of observation per hotel, which would correspond to a provisional total minimum sample size of  $N = 168 \text{ days} \times 4 \text{ hotels} = 672 \text{ days of observations}$ .

### 5.2.8. Statistical power estimation

Considerable complexity is associated with this type of field trial, including the existence of fixed (e.g., treatment) and random effects (site and number of observations and replicates) and possible hierarchical dependence between observations across sites over time. To account for all factors, the final sample size and statistical power needed for the study were estimated using simulations, following the approach proposed by Bolker (2007). The statistical power was estimated considering that an initial minimum number of 168 replicate-days/site would be enough to detect a minimum reduction of 40% (effect size) in relative risk of mosquito abundance at treated hotels (intervention arm) compared to untreated (control arm) hotels. The effect size was set based on findings from a larger and multicentric larval source management randomized trial conducted in Nicaragua and Mexico (Andersson et al., 2015). Accordingly, the overall mean variation of mosquito counts between seasons, daily replicates and hotels were estimated according to a previous survey at the same hotels as being, respectively, 18.5, 2, and 5 (Chapter 2 and Chapter 3). The daily mosquito counts were assumed to follow a negative binomial distribution with overdispersion in mosquito counts of  $k = 0.5$ . This information was applied to simulate the expected spatiotemporal distribution of mosquito counts according to type of intervention. For this particular purpose, Hotel A and Hotel B were arbitrarily considered as being treated, whereas Hotel C and Hotel D functioned as control. The generated dataset with factors was then used to fit a probable effect of interventions on simulated mosquito counts (i.e., response variable) by using generalized linear mixed models (GLMM) at  $\alpha = 5\%$  significance level. The resulting model was iteratively fitted 1000 times by means of Monte Carlo simulations via the R software package *simr* v. 1.0.5 (Green & MacLeod, 2016). Here we gradually increased the number of observations within hotels and extracted the  $p$  – values from fitted models (see Fig. 5.2). Models were fitted to address the hypothesis that there would be no significant reduction in overall mosquito counts in the intervention arm compared to the control. The mean proportion of the time this null hypothesis was rejected is then the

statistical power (Bolker, 2007; Johnson et al., 2015). Simulation results suggest that at least 142 sampling days are required per site. This would confer a satisfactory statistical power of 81% (CI = 79% – 83%) (Figure 5.2).

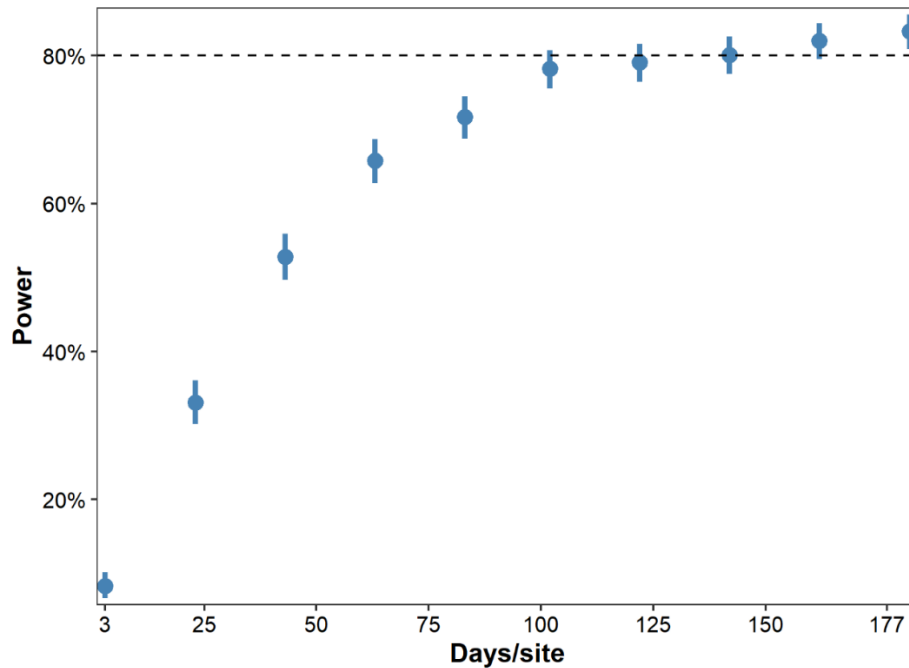


**Figure 5.2.** Inferred sample size required to detect significant variations of mosquito abundance associated with introduction of larval sources management interventions at hotels. A minimum 142 replicate-days of observations are required at each of the four experimental sites.

## 5.2.9. Data collection procedures

### 5.2.9.1. Characterization of mosquito larval sources

In each hotel, mosquito surveys will be undertaken for seven days per month over 142 days. Outdoor spaces of the hotel compounds will be inspected for the presence of immature mosquito stages. Only containers holding water will be examined to record the presence of immature mosquitoes. Mosquito larval sources will be divided according to type (i.e., natural and artificial containers) and sub-classified according to physical characteristics, function, location and dimension (Table 5.1), as suggested by Flaibani et al. (2020) and used in our previous findings (Chapter 3).

**Table 5.1.** Proposed scheme for characterization of containers supporting the development of mosquito larvae according to physical and functional features, and location in the landscape. Adapted from Flaibani et al., (2020) and Kampango et al., (2021a) (Chapter 3).

| Characteristic | Categories        | Description                       |
|----------------|-------------------|-----------------------------------|
| Physical       | Type              | Cans/Tins                         |
|                |                   | Bottles                           |
|                |                   | Buckets                           |
|                |                   | Dishes                            |
|                |                   | Flowerpots                        |
|                |                   | Jars                              |
|                |                   | Fast food containers              |
|                |                   | Swimming pools                    |
|                |                   | Tanks                             |
|                |                   | Wells                             |
| Physical       | Material          | Coconut shells/Flower spathes     |
|                |                   | Mollusc shells                    |
|                |                   | Tree holes                        |
|                |                   | Fallen leaves                     |
|                |                   | Tyres                             |
|                |                   | Plastic                           |
|                |                   | Clay                              |
|                |                   | Metal                             |
|                |                   | Rubber                            |
|                |                   | Concrete                          |
| Physical       | Size/Volume       | Glass                             |
|                |                   | Small (< 1litres)                 |
|                |                   | Medium (>1 litres and <5 litres)  |
|                |                   | Large (> 5 litres and <10 litres) |
|                |                   | Very larger > 10 litres           |
| Function       | Purpose           | Gardening                         |
|                |                   | Water storage                     |
|                |                   | Animal feeding                    |
|                |                   | Recreation                        |
| Function       | Use               | In use                            |
|                |                   | Not in use                        |
| Location       | Environment       | Staff room quarter                |
|                |                   | Guest room quarter                |
|                |                   | Administrative area               |
|                |                   | Restaurant/Bar area               |
|                |                   | Dumpsite                          |
|                |                   | Kitchen/Laundry area              |
|                |                   | Garden/Open spaces                |
|                |                   | Plant nursery                     |
|                | Sunlight exposure | Not exposed                       |
|                |                   | Exposed                           |

#### 5.2.9.2. Immature mosquito sampling strategy

The following sampling strategy has been adapted from Knox et al. (2007), Manrique-Saide et al. (2011) and Tun-Lin et al. (1994a) for the collection of mosquito larval data, depending on container size and water volume. For containers of less than 20 litres of water (e.g., bottles, snail shells, flasks, tins, tyres, tree holes, and coconut husks, etc.), all the larvae and pupae are collected by sieving the water directly through a mesh net or by a pipette. If water volume is more than 20 litres and emptying the container is not feasible (due to its size or nature of the container) but there is good visibility (tank/drums, pots, swimming pools, tubs, etc.) and low larval and pupal density (less than 100 individuals), then all the specimens are sampled using dippers and sweep nets (Dinglasan et al., 2009). Otherwise, only 10 - 15 dips/sweeps are taken at random to sample immature stages and then a correction factor is applied to estimate density. If containers are too deep (e.g., underground wells), samples are collected using small buckets (approx. 5 litres) suspended by a rope. Artificial containers found with water, but no larvae (on the day of sampling) are considered as potential mosquito larval sites if they have physical conditions to retain collected water for at least three consecutive days. Samples are marked according to collection day and location and transported to an insectary for further processing. Larvae of mosquito species that are known to be predacious, such as, *Toxorhynchites* species, *Cx. tigripes* and *Eritmapodites* species, need to be separated into different vials to avoid predation on other mosquito larvae. An extra sample of prey mosquito larvae is collected to feed the predacious ones.

#### 5.2.9.3. Adult mosquito sampling strategy

A survey of adult mosquito populations will also be undertaken concurrently with larval surveys to assess whether any impact on immature mosquito stages is translated into lower adult mosquito populations and, consequently, reduction in bite exposure. Therefore, outdoor host-seeking mosquitoes will be sampled for 24-hours using BG Sentinel traps (Biogent, Germany) baited with CO<sub>2</sub>. Outdoor resting mosquitoes will also be collected using Prokopack aspirators (John W. Hock Company, USA) between 06:00 - 09:00 hours.

#### 5.2.9.4. Sample processing and identification

Following the procedure described in Chapter 2, collected samples of immature mosquitoes will be reared under insectary environmental conditions of temperature ( $27 \pm 2^\circ\text{C}$ ) and relative humidity ( $75 \pm 10\%$ ) until they emerge as adults (WHO, 2016). Larvae will be fed either with

ground adult cat food biscuits (non-*Anopheles* mosquito larvae) or with Koi fish food (*Anopheles* larvae) and kept at a 12:12h light:dark regime until adult emergence. Adult mosquitoes will be euthanised by placing the specimens inside a refrigerator. Dead mosquitoes will be grouped in batches of ten to twenty specimens, and then transferred into Eppendorf tubes containing silica gel for morphological identification. Morphological identifications will be done using taxonomic keys for Afrotropical mosquito fauna (Coetzee, 2020; Edwards, 1941; Huang, 2004; Jupp, 1986; Service, 1990). Mosquitoes that cannot be identified morphologically to species, such as members of species complexes, will be placed individually in Eppendorf tubes with silica gel and store at -20° C for further molecular analysis.

#### **5.2.10. Monitoring and evaluation of intervention impacts**

Potential impacts of the interventions will be observed monthly during the trial period. The monitoring and evaluation process aim to measure the trend of the following entomological indicators in both control and intervention arms, namely: mosquito immature occurrence and abundance, and type, frequency, and productivity of larval habitats. For *Aedes* mosquitoes, some *Aedes* (*Stegomyia*) immature indexes, namely container index and pupae will also be assessed. Since consistent reduction of mosquito larval stages is not itself a sufficient indicator of the success of control interventions (WHO, 2013), the impact of the interventions on adult mosquitoes occurrence and abundance also needs to be measured.

#### **5.2.11. Quality assurance assessment of control operations and staff performance**

Periodic evaluation of the quality of application and execution of mosquito control operations by trained field staff by a senior supervisor or an independent group of assessors is needed. This ensures that interventions have been applied correctly and at the appropriate time, whether control operation staff work in designated areas, that habitats have been searched for immatures and adult mosquitoes, and that the interactions between control staff, hotel staff and hotel guests support the goals of the project. This information will be shared with project coordinators for analysis and implementation of any corrective measures that are required.

#### **5.2.12. Data analysis**

Joint species distribution models (JSDMs) are appropriate for evaluating the response of mosquito communities to intervention (Warton et al., 2015). The JSDMs will be fitted using the Bayesian hierarchical model of species community (HMSC) approach proposed by

Ovaskeine et al., (2017). Changes in both mosquito community occurrence and abundance in response to intervention can be assessed with these models. JSDMs will be fitted using the software Hmsc v. 3.0-12 (Tikhonov et al., 2020). For modelling the mosquito community occurrence, mosquito counts will be converted into a presence ( $Y=1$ ) and absence ( $Y=0$ ) binary matrix. The presence-absence observation will be assumed to follow a probit distribution. For the abundance model, abundance conditional on presence modelling approach will be applied. To do so, count data is transformed by declaring zeros as missing data, log-transformed, and then scaled to zero mean and unit variance for each species. Since modelling abundance requires more information, it is proposed that a subset of species that were observed in at least ten sampling occasions be used to model the impact of the intervention on abundance. A similar approach has been applied elsewhere (Ovaskaine & Abrego, 2020). Together with the presence or absence of interventions, season will also be added to the models as a fixed effect for its significant effect on mosquito occurrence and abundance as described in previous surveys at same sites (Kampango et al., 2021a; Kampango et al., 2021c). Site and days of survey will be included in the models as community level random effects related to spatial and temporal variations in mosquito occurrence and abundance. Additionally, the models will be phylogenetically corrected to explore whether closely related species have similar responses to the interventions than distantly related species. The phylogenetic tree used in the analyses will encompass six levels, ranging from mosquito species order, family, subfamily, genus, subgenus, and species and be derived from data published by (Harbach, 2013). The R software package ape v. 5.5 will be used to convert the taxonomic tree into a matrix, assuming equal branch lengths among families, among genera within a family, and among species within a genus (Ovaskaine & Abrego, 2020). All the statistical analysis will be performed using the latest available version of the open source domain software R (R Core Team, 2022).

### **5.2.13. Data management**

Standard field data collection methods will align with those used in previously published observational studies (Kampango et al., 2021c). The collected data will include mosquito larval habitat type, applied interventions, presence and absence of mosquito larvae, and number of visited sites. Approved data will be entered into password secured Excel spreadsheets for future analysis and preparation of feedback reports to project coordinators and partners. This study is not a clinical trial evaluating a therapy with mortality or irreversible morbidity endpoints, so no data safety monitoring board is needed.

#### **5.2.14. Primary outcomes**

The primary outcomes of the project will be the efficacy of interventions on mosquito occurrence, abundance, and composition of mosquito assemblages in the intervention arm compared to the control arm.

#### **5.2.15. Secondary outcomes**

Secondary outcomes of the project will be the characterization of larval habitats, according to type, function, and location as detailed in Table 5.1.

#### **5.2.16. Advocacy and communication**

Prior to implementation of the study, a communication plan will be produced, in collaboration with other partner institutions, to attract support from the local community and relevant actors/stakeholders including the Zanzibar Association of Tourism Investors (ZATI), environmental authorities, and Zanzibar Ministry of Health through the ZAMEP. ZAMEP and other identified institutions, as well as non-Governmental Organizations (NGOs), involved in policy making, decision-making and implementation of MBD control interventions in the archipelago will be approached to identify options for collaboration and sharing of resources to improve efficiency and optimize benefits for public health.

#### **5.2.17. Environmental impact assessment**

Environmental management interventions are expected to reduce the impact on the environment of vector control (WHO, 1982). However, some operations may affect certain non-target organisms. Therefore, prior to implementation of interventions, consultation with experts from Zanzibar Environment Management Authority (ZEMA) will be needed to identify any potential protected plants or animals occurring within the studied areas and get advice on how to safeguard the integrity of the organism in question. Alternatively, if no specialist is found, an environmental impact assessment matrix (WHO, 1982) will help to identify and minimise potential non-target effects.

### **5.3. Ethical considerations**

Ethical clearance for the proposed trial has been granted by the Zanzibar Health Research Ethics Review Committee (ZAHREC), Ref: No. ZAHREC/03/PR/Oct/2019/001, in the Second

Vice President's Office. Permission to undertake the trial has also been obtained from the hotel managers at each hotel, in the form of signed consent letter. Additional permission to undertake the study will be sought from local community leaders (*Shehia*) responsible for the area where the study will be conducted. Accordingly, community members will be informed about the purpose of the trial and their opinions collected and incorporated where applicable. For privacy reasons, hotel names will be replaced by a code in the data collection forms and in any eventual reports.

#### **5.4. Field assessment of the framework**

Field experiments to assess the feasibility and impact of proposed interventions were planned to be carried out within the time allocated for the PhD studies (2019 – 2022). However, the framework did not move on to field testing due to global restrictions imposed by the COVID-19 pandemic which had dramatically affected the tourism industry in Zanzibar, forcing hotel partners closed doors for over a year (2020 – 2021). However, there are intentions to evaluate the framework in the future, possibly as part of a potential BSc or MSc student degree.

#### **5.5. Discussion**

With the advent of DDT, global efforts to control MBDs by mean of environmental management approaches shifted radically to the widespread use of synthetic chemical insecticides (Achee et al., 2015). However, emergence and widespread resistance in vector species worldwide, coupled with resurgence of MBDs in regions where it had earlier been eradicated is a clear reminder that effective elimination of MBDs may not occur by relying only on stand-alone insecticide-based interventions. In fact, historical (Andersson et al., 2015; Keiser et al., 2005; Konradsen et al., 2004; Lindsay et al., 2004; Takken et al., 1990; Trewin et al., 2017b) and contemporary evidence (Castro et al., 2009; Maxwell et al., 1990; Whelan et al., 2020) show that effective MBD elimination programmes were achieved by integrated implementation of vector control approaches that relied fundamentally on mosquito larval source management interventions.

The main factors that have discouraged wide-scale use of environmental management interventions (EMI) for vector control are the initial implementation costs and the challenges related to scaling-up interventions to cover large areas in a short timeframe (van Emden & Service, 2004). However, systematic analysis has shown that EMI can be economically cost-effective in the long-term due to the longevity of its protection (Keiser et al., 2005; Worrall &

Fillinger, 2011). Moreover, it can reduce the likelihood of selection for insecticide resistance (Keiser et al., 2005; WHO, 1982; 2012). Perhaps two of the most notorious cost-effectiveness analyses of EMI conducted in the sub-Saharan region come from studies on malaria control in copper mines in Zambia (Utzinger et al., 2001) and at a military base in Apapa, Nigeria (Gilroy & Chwatt, 1945). In Zambia, the full package of control measures consisted of vegetation clearance, modification of riverbanks, draining swamps, oil application to open water bodies and house screening (Utzinger et al., 2001), whereas in Nigeria it consisted of swamp drainage (Gilroy & Chwatt, 1945). Malaria incidence was reduced by 70% to 95% in Zambia and Nigeria, respectively, after the introduction of these control measures. Moreover, the interventions averted 4,173 deaths and 161,205 malaria attacks in the Zambia study. EMI can thus control a wide range of vectors and nuisance pests sharing the same breeding sources. Evidence with malaria vectors control interventions have shown that although initial cost of environmental management may be high, particularly during the installation of infrastructure, long term maintenance costs can be exceptionally low while the efficacy of control measures can prevail for decades (Keiser et al., 2005). Moreover, environmental management may easily receive community support for eliminating not only a small segment of disease vectors but also by reducing the overall biting rate by nuisance pests (WHO, 2012; 2013).

The protocol that we propose will assess the impact of integrating larval source reduction and installation of water dispensers on vectors and nuisance mosquito species at hotel compounds in Zanzibar. We anticipate that combining these types of intervention is more likely to cause meaningful reduction of vector species than if each was evaluated separately. This innovative approach is less environmentally destructive than other EMI interventions and may help to break the loop of production of single-use plastic water bottles that are discarded and eventually become mosquito larval habitats at hotel compounds (Kampango et al., 2021a; Kampango et al., 2021c). However, the success and sustainability of this type of hotel-based mosquito management approach depends on the compliance of studied hotels. Therefore, to minimize the risk of non-compliance, and its potential impact on mosquito occurrence and abundance, we suggest that the intervention is performed in daily coordination with designated hotel staff members. Another challenge this type of study would face is the establishment of buffer zones, since some hotels are immediately surrounded by either other hotel compounds, human settlements, or by nearly impenetrable forested areas. Therefore, extending treatment to such areas may not be feasible.

Our protocol involves both contemporary (phase I) and historical (phase II) controls. This is expected to reduce the between-hotel variability, permitting the detection of smaller effect-sizes (in this case reduction in mosquito probability of occurrence and abundance) with relatively low investment in resources (Hutson & Reid, 2004; Stoney & Johnson, 2018). Moreover, partial cross-over design can have similar efficiency as a complete cross-over design in answering complex questions asked in a feasibility (phase II) trials proposed here (Hutson & Reid, 2004). Results from this trial will support a decision-making process exercise and determine whether to proceed to large scale phase III trial.

Worldwide, tourism destinations have been confronted by an unprecedented plastic crisis due to proliferation of single use plastic water bottles (Kapmeier & Gonçalves, 2018). This has led to calls for global sustainable solutions that reduce or totally replace disposable plastic bottles. This has also been acknowledged in the Zanzibar Blue Economy Policy (The Revolutionary Government of Zanzibar, 2020). Perhaps one of the most cost-effective sustainable approaches that has received attention in the tourism industry are onsite water refilling practices (EHL Insights, 2020; Loding Magazine, 2019). In Thailand, implementation of onsite water refilling policies in a complex of hotels, resorts and spas has prevented the disposal of 1.8 million single-use plastic water bottles per year (Six Sense, 2021). The impact of type of policy change on container-breeding mosquito population has yet to be tested addressed. This protocol can be the base for future studies aimed to evaluate the impact of environmental control interventions on mosquito risk exposure at hotel compound settings.

## **5.6. Acknowledgements**

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## CHAPTER 6

### General discussion and conclusion

The work presented in this thesis expands our knowledge on the ecology and biology of Africa mosquito population vectors of viral agents of global health concern. It characterized the ecological and environmental factors driving the occurrence, distribution, and persistence of mosquito vectors within the hotel settings of Zanzibar. This information is then used to develop a framework to further support the design of a vector control efficacy trial involving environmental management interventions and implementation of a comprehensive vector surveillance system in Zanzibar to improve preparedness for re-emergence of mosquito-borne viral epidemics in the region (Mweya et al., 2016; Saleh et al., 2020a). Climate and environmental changes, high connectivity between Zanzibar and surrounding endemic regions, rapid demographic expansion, and high mosquito infestation are arguably the possible drivers of future MBDs epidemics (Kampango et al., 2021c; Kukkonen et al., 2018; Mwalusepo et al., 2017; Mweya et al., 2016; Saleh et al., 2020a).

The main research questions explored in this work were: 1) What are the structure, dynamics, and drivers of mosquito community assemblages at hotel compounds in Zanzibar? 2) What are the structure and dynamics of mosquito-larval habitat interaction networks, and the degree of robustness against larval habitat removal at hotel compounds? 3) What are the environmental drivers of dominant mosquito vector species occurrence and abundance at hotel compounds? 4) What are the predominant mosquito control practices at the hotel compounds? 5) What is the status of insecticide susceptibility in the dominant mosquito vectors species at hotel compounds? The main questions and key findings are reported in Table 6.1.

**Table 6.1.** Overview of the main research questions, findings, and conclusions. The numbers refer to the chapter in which the question has been presented in detail.

| Question                                                                                                                                              | Chapter | Key findings and conclusions                                                                                                                       |                                                                                                                   |                                                                                                                                             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|---------|----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| What is the structure, dynamics, and drivers of mosquito assemblages at hotel compounds?                                                              | 2       | Twenty-six mosquito species were identified from pooled immature and adult mosquito catches.                                                       | Network structure analysis revealed the presence of abundant generalist species and rare specialist species.      | Plastic containers are keystone larval habitat resources.                                                                                   |
| What is the structure of mosquito – larval habitat interaction networks and the degree of robustness against larval habitats loss at hotel compounds? |         | Mosquito abundance peaks during the long rainy season, whereas species diversity peaks one to three months after the end of the long rainy season. | Degree of specialization is season-dependent, with <i>Aedes aegypti</i> representing the most generalist species. | The mosquito community structure is least tolerant to elimination of plastic containers.                                                    |
| What are the environmental drivers of <i>Aedes</i> mosquito vector occurrence and abundance at hotel compounds?                                       | 3       | <i>Aedes</i> mosquitoes of major global health concern identified.                                                                                 | <i>Aedes aegypti</i> and <i>Aedes bromeliae</i> are dominant <i>Aedes</i> vector species.                         | <i>Aedes</i> occurrence and abundance are sustained by the presence of poorly managed solid wastes and containers used for gardening tasks. |
| What are the predominant mosquito control practices at hotel compounds?                                                                               | 4       | Indoor and outdoor space spraying and fumigation are the two most frequent mosquito control practices.                                             | Organophosphates, pyrethroids and carbamates among the most used insecticides.                                    | Insecticide spraying is generally performed biweekly without oversight from authorities.                                                    |
| What is the status of insecticide in the dominant vector species at hotel compounds in Zanzibar?                                                      |         | <i>Aedes aegypti</i> is resistant to DDT (at all hotel sites) and deltamethrin (at some sites).                                                    | Knockdown resistance mutation V1016G identified but association with phenotypic resistance unresolved.            | Further investigations are needed to understand the causes and consequences of the emergence of V1016G resistance allele in Zanzibar.       |

**Objective 1:** To investigate the structure, dynamics, and environmental drivers of mosquito population assemblages at hotel compounds in the southeast coastal region of Zanzibar (Unguja) Island.

The spatiotemporal dynamics of MBD transmission is largely determined by complex interactions between climatic and environmental factors. Variation in these factors influence the structure of vector and pathogen traits, and, eventually, disease transmission (Ellis & Wilcox, 2009; LaDeau et al., 2015). For instance, fluctuation in temperature, rainfall and humidity modulate vector and pathogen development rate, survival, longevity, biting activity, and pathogen proliferation inside the vector (Mordecai et al., 2019; Shocket et al., 2021). Likewise, anthropogenic environmental changes, mostly deforestation, have increased human vulnerability to MBDs by providing vectors with new habitats and increased host ranges (Sutherst, 2004). Since the dynamics of climatic and environmental factors are essential for sustained MBDs transmission (Shocket et al., 2021), thorough understanding of how these factors promote or limit vector species assemblages and contact with human hosts is vital for the design of effective vector management strategies.

In this study, I found that fluctuations of mosquito abundance, composition and diversity closely follow rainfall patterns. High mosquito abundances were observed during the wettest months of the long and short rainy seasons, that is, May and December, respectively. Likewise, mosquito community diversity indexes also peaked in association with rainfall, albeit with a delayed response. Mosquito species diversity tended to peak one month after the end of the rainy seasons, and in some hotels, diversity remained higher for three consecutive months (Chapter 2, Figure 2.3). These findings suggest that the mosquito community reaches stability one to three months after rainfall ceases. Time-lagging rainfall effects have also been observed elsewhere in several other mosquito taxa (Caldwell et al., 2021; Chang et al., 2016; Franklin & Whelan, 2009), indicating that vector exposure at the beginning of the dry season may be as high as in rainy season. This must be considered when setting the epidemiological thresholds signifying the beginning and end of the transmission season in studied regions. Moreover, the findings have direct operational implications for the design and implementation of vector management interventions.

**Objective 2:** To determine the structure and dynamics of larval habitat utilization networks by mosquito vector populations.

Mosquitoes interact with abiotic and biotic resources for reproduction and survival, as outlined in the literature review (Chapter 1) and reaffirmed by the findings of this thesis (Chapter 2). The magnitude of connectivity, dependence, and intraspecific and interspecific competition for these resources shapes the community structure and persistence of vector species across landscapes. Management of mosquito abiotic resources, most specifically larval habitats, is a core component of integrated vector management (Beier et al., 2008). It is arguably one of the more cost-effective and cost-saving vector control alternatives for densely inhabited settings, where satisfactory delivery and coverage of convectional interventions (IRS and LLINs) is extremely challenging or not operationally feasible (Worrall & Fillinger, 2011). One of the biggest challenges in implementing larval resource management is to determine how mobile organisms such as mosquitoes use a range of available resources across multiple environments. Likewise, knowing the patterns of specialization in habitat selection by the entire community is even more challenging due to the inherent heterogeneity of landscapes and number of species involved (Lami et al., 2021). Therefore, thorough knowledge of how species utilize resources across space and time is pivotal for the implementation of effective larval source management strategies.

An ecological interaction network (EIN) is a powerful tool that has helped to elucidate some of the complexities in the interaction of different species and their habitats (Cappellari & Marini, 2021; Lami et al., 2021; Marini et al., 2018). Surprisingly, this approach has been overlooked when investigating key environmental resources that drive vector community responses to environmental disturbances such as habitat fragmentation or modification, and eventually risk of disease transmission. To the best of my knowledge this is the first attempt to apply EIN tools to characterize the dynamics and patterns of larval habitat utilization by different mosquito populations. By doing so, I was able to demonstrate that the mosquito community at the studied sites comprises assemblages of abundant generalist species that share larval habitat resources with a group of less abundant specialist species (Chapter 2). This type of nested interaction network is usually observed in mutualistic networks (Bascompte, 2010), and could possibly provide community robustness against disturbances (Bascompte, 2010). Furthermore, I also identified plastic bottles as a keystone resource that maintains mosquito species diversity at the studied sites.

Therefore, I suggest that implementation of larval source management strategies at studied hotels should target the production of disposable plastic bottles within hotel compounds and in the surrounding areas.

**Objective 3:** To investigate the robustness of mosquito communities to designated larval source elimination strategies.

There is a need to reinstate long overlooked environmental mosquito management practices to tackle vector species and the widespread problem of insecticide resistance development. In fact, vector control programmes have been encouraged to develop integrated vector management strategies with environmental control as a fundamental component (WHO, 2012). However, one of the greatest challenges with environmental measures is the lack of tools to infer whether physical environmental intervention measures (e.g., larval source removal) produce the desirable effect against local mosquito fauna. Addressing this question is critical given the high initial costs associated with implementing environmental management trials. For chemical and biological control approaches, *in vivo* susceptibility bioassays can give robust information to support the selection of appropriate insecticide classes or biological agents. However, there is no similar robust laboratory approach for assessing larval habitat removal. Therefore, we propose that EIN tools can be used to make predictions of the most probable response caused by targeted larval source removal (LSR) that eliminate mosquito larval habitats. Specifically, the EIN approach can be used to perform simulations on field collected data to predict the effect of realistic LSR strategies, and thereby avoid wasteful use of limited resources.

By applying EIN analysis, I was able to predict the most probable responses of mosquito communities at study sites by determining their robustness against three potential types of LSR strategies. Notably, I predicted that control measures that target the most connected larval habitats sustaining high mosquito species diversity would be more effective than uniform or indiscriminate removal of larval habitats. Therefore, I argue that analysis of network robustness can be an important tool to perform *in-silico* assessment of the “susceptibility” of mosquito populations to specific LSR approaches. As such, this tool can provide invaluable information for the decision-making process in MBD control. However, longitudinal LRS field trials are encouraged to test the

hypothesis that elimination of keystone larval habitats will result in significant reductions in mosquito populations and disease transmission threats.

**Objective 4:** To determine local environment associated factors promoting occurrence and abundance of the dominant vector species, *Aedes aegypti* and *Ae. bromeliae*, at hotel compounds.

At least fifty-nine species and subspecies of the genus *Stegomyia* are recognized in the sub-Saharan region (Huang, 2004). Among them, *Ae. aegypti* is the traditional and most widespread vector of DENV, CHIKV, and YFV in the region (Huang, 2004). Evidence also indicates that *Ae. bromeliae* is one of the most important vectors of YFV, especially in eastern regions of the continent (Huang, 1986). For instance, a recent study from Kenya reported a high survival rate and human blood index in *Ae. bromeliae* populations, which may also suggest high vectorial capacity for pathogens (Kamau et al., 2022). Another study, also conducted in Kenya, suggests that *Ae. bromeliae* may be the most important vector of YFV in urban areas (Agha et al., 2022). In Zanzibar, the vector status of *Ae. aegypti* and *Ae. bromeliae* has yet to be confirmed. However, occurrence of these competent vectors in highly human frequented locations, such as hotel compounds, underscores urgent need to implement comprehensive area-wide assessments of *Aedes*-borne arbovirus transmission risk. The hotel-based risk assessment of *Aedes* vectors reported in this thesis (Chapter 3) shows the occurrence and abundance of *Aedes* mosquitoes. Notably, the most prevalent vector species *Ae. aegypti* and *Ae. bromeliae* are favoured by human activities such as production of poorly disposed solid wastes, and most importantly, plastic and metal containers that function as larval habitats. Nevertheless, some natural containers, such as tree holes and coconut shells also play an important role as mosquito larval sources in the rainy season. Given the types and nature of larval habitats comprising largely small containers, my findings are a clear indication that implementation of mosquito control intervention by chemical or biological larviciding would not be feasible. Treatment of a huge number of small containers is logistically unrealistic, since conventional larvicides require retreatment of larval habitats on a weekly or biweekly basis to maintain residual efficacy (Coosemans & Carnevale, 1995). Moreover, the timing of treatment usually depends on container size and the amount of dissolved organic matter (Coosemans & Carnevale, 1995). As such, I propose that implementation of integrative measures to completely remove larval habitats and halt production chains of artificial and natural containers that eventually end up in the environment may effectively control mosquitoes at study sites.

**Objective 5:** Outline existing mosquito control practices, determine the status of insecticide susceptibility and molecular mechanisms underpinning DDT/pyrethroids in *Ae. aegypti* populations at hotel compounds.

The aim of mosquito control interventions is to reduce vector populations to a level at which they no longer constitute a public health threat (WHO, 2014). However, deployment of interventions should be based on the integrated use of a range of interventions selected on the basis of local knowledge about targeted vector populations ecology (WHO, 2012). Use of synthetic insecticides has spearheaded worldwide vector control campaigns (Whalon et al., 2008). However, the widespread evolution of resistance means that insecticide application for vector control may become the last resort (WHO, 2012). Therefore, knowing insecticide use practices and status of insecticide susceptibility in vector species is critical for safeguarding the efficacy of the limited insecticide options available for public health use. This information may prevent wasteful use of ineffective insecticides (Georghiou, 1994) and can help to establish the window of selection, that is, the timing between insecticide introduction and the emergence of resistant strains in the population (South et al., 2020).

My project has revealed that the investigated hotels make intense use of insecticides, mostly carbamates, organophosphates and pyrethroids. Importantly, the choice of insecticides is not supported by susceptibility assays. Therefore, indiscriminate use of insecticides may be causing focal selection for resistance against several groups of insecticides even before wide-scale introduction by public vector control programmes. This may seriously jeopardize the sustainability of resistance management adopted by Zanzibar health authorities. Remarkably, the hotel sector is not covered by public vector control campaigns and hotels often use commercial companies for mosquito and, generally, pest control. A lack of oversight of the commercial sector may also contribute to uncritical application of insecticides in hotel settings.

In my susceptibility bioassays, I showed the presence of phenotypic resistance to DDT in all four hotels, whereas resistance to pyrethroids was obvious only in some hotels. This represents the first attempt to describe the insecticide resistance profile of *Ae. aegypti* populations in Zanzibar. Previous surveys in the mainland region of Ifakara reported full susceptibility to DDT and pyrethroids (deltamethrin and permethrin) in local *Ae. aegypti* populations (Kahamba et al., 2020).

Another study in Dar es Salaam reported resistance to deltamethrin and susceptibility to permethrin (Mathias et al., 2017). However, the magnitude of resistance observed in my bioassays could not be consistently linked to the presence of knockdown resistance genes nor to glutathione-S-transferase gene mutations, associated with resistance to pyrethroid and DDT respectively, given the very low frequency of *kdr* and *GSTE2* gene polymorphisms. My finding implies that mutations in these genes may not play a role in observed DDT and pyrethroid resistance. Instead, the observed resistance might be driven by metabolic mechanisms. Similar findings have been observed in *Ae. aegypti* population in Congo (Kamgang et al., 2020) and Senegal (Sene et al., 2021).

Vector control using DDT in Zanzibar was discontinued 40 years ago, due to widespread resistance in the malaria vector *An. gambiae* s.l. (Schwartz et al., 1997). Therefore, the causes of current DDT resistance observed in all four sites remains a mystery. Gene expression analysis may provide insights on probable metabolic resistance mechanisms involved. However, it has been convincingly shown that mosquitoes can retain resistance to DDT for a long period after use of the insecticide has been terminated (Raghavendra et al., 2010). In some cases, for *Anopheles* species, reversion to susceptibility was observed many years after DDT pressure was removed (Abedi & Brown, 1960; Bang et al., 1971). However, it is worth noting that susceptibility restoration has been observed repeatedly with insectary maintained resistant strains of *Ae. aegypti*, after rearing the mosquitoes in the absence of insecticide pressure for ten generations (Bisset et al., 2020; Grossman et al., 2018; Thornton et al., 2020).

The occurrence of the *kdr* resistance polymorphism V1016G, although at lower frequency, is of concern as this type of mutation has been shown to confer resistance to both type I and type II pyrethroids (Djiappi-Tchamen et al., 2021; Moyes et al., 2017). Therefore, operational implications of the presence of this type of mutation may be noticed in the near future, unless actions are taken to mitigate the spread of this type of polymorphism. The discovery of this mutation may also open avenues of investigation on evolution and expansion of emerging genetic polymorphism associated to DDT/pyrethroid cross-resistance in Tanzania.

**Objective 6:** To develop a tool for implementation of hotel-based mosquito larval source reduction interventions and assessment of mosquito risk exposure at hotel compounds in Zanzibar.

Development and implementation of vector management interventions is a context-specific and evidence-based decision-making process, supported by accurate baseline information about vector and disease determinants (WHO, 2012). However, in many cases, the tools for assessing the efficacy of novel control measures are usually non-existent, particularly for vector species other than malaria vectors (WHO, 2017b). Environmental management has repeatedly and convincingly helped control and even eliminate MBDs, most notoriously malaria and dengue (Buhler et al., 2019; Keiser et al., 2005; Killeen et al., 2002; Takken et al., 1990; Toledo et al., 2011; Watson, 1911). However, as poorly designed and conducted studies reduce the certainty of the evidence, robust experimental designs are needed to provide strong evidence of causality (WHO, 2017b).

Based on the findings of this project, I propose a research approach for integrated evaluation of the efficacy of larval habitat removal and onsite water refilling practices on mosquitoes at hotel compounds. This integrated environmental management approach is novel in the sense that it has the potential to simultaneously reduce plastic waste and remove larval habitat sources, with plastic water bottles comprising approximately 70% of all container types that support larval development (Chapter 3). Recently implemented water refilling practices at other hotel facilities (EHL Insights, 2020; Loding Magazine, 2019) have reduced the use and disposal of over one million plastic bottles per year that could have potentially ended up in the environment (Six Sense, 2021). To the best of my knowledge, the entomological impact of policies for plastic water bottle reduction has never been evaluated before in a field trial. Regretfully, the COVID-19 pandemic forced all four hotels to close temporarily, only resuming business nearly two years later (2020 – 2021). Consequently, we were unable to perform any field evaluation of proposed interventions within the allocated PhD period (2019 – 2022). However, the proposed trial protocol may hopefully inspire the design and evaluation of the suggested mosquito control intervention in similar trial contexts.

## Limitations, future directions, and outstanding research needs

Further studies are encouraged to measure the risk potential of arbovirus transmission at hotel facilities. My findings enhance knowledge of the ecology and biology of mosquito vectors occurring in Zanzibar and set the foundations for further investigations on transmission ecology of mosquito-borne viral diseases in the region. Importantly, my findings underscore the need to consider hotels in future outbreak risk assessments and vector surveillance activities in Zanzibar. However, as the study was limited to hotel compounds and their immediate surrounding, the thesis conclusions cannot be generalized to communities beyond the hotel sector. Therefore, further studies are required to address some outstanding questions namely:

Do mosquito community structures and patterns of ecological interactions with larval habitats differ between hotel compounds and surrounding human settlements? To what extent can hotels function as hotspots of residual vector species that escape control measures in adjacent communities? Can hotel-based mosquito communities be considered a subcommunity of mosquito communities from surrounding settlements? How far can mosquito vectors disperse from hotel compounds? To what extent do *Ae. aegypti* populations in these communities remain susceptible to chemical insecticides? Has the knockdown resistance mutation allele found in *Ae. aegypti* at Hotel B emerged in this hotel or spilled over from surrounding settlements? How has the V1016G *kdr* mutation spread in Zanzibar?

Answering these questions are critical for the design of operationally sound vector control strategies and to the implementation of regional insecticide resistance management plans.

## Conclusions

Mosquito-borne diseases continue to postpone the societal and economic progress of endemic countries. Efforts to restrict transmission need to rely on vector control interventions and the collection of comprehensive, reliable information on vector exposure and disease epidemic risks. My findings demonstrate that hotels in Zanzibar provide a suitable environment for production and maintenance of highly competent mosquito vectors, notably *Aedes aegypti*, *Ae. bromeliae*, *Ae. vittatus*, *Ae. africanus*, and *Eretmapodites chrisogaster*. Mosquito communities are sustained by locally generated and improperly disposed artificial and natural containers, as well as containers

used in gardening tasks. Open spaces and residential areas allocated to hotel staff within the hotel compounds are associated with high mosquito production.

The structure and dynamics of mosquito communities follow the seasonal pattern of rainfall, with higher mosquito abundance occurring during the rainy season, whereas species diversity peaks at the beginning of the dry season. This finding implies that the end of the rainy season may not necessarily reduce mosquito exposure. Fluctuations of vegetation cover also modulate mosquito abundance and diversity. High vegetation cover means higher mosquito abundance and diversity at studied sites. Ecological interaction network (EIN) analysis revealed that mosquito community structures are characterized by the presence of generalist species that utilize a great proportion or virtually all types of available water holding containers as larval habitats. Community structures also include specialist species that colonise single types or a relatively narrow range of containers. Controlling generalist species would require greater logistical efforts compared to specialist species, as a generalist species may be able to replace lost larval habitat resources by any other type of container available. The degree of specialization is season-driven, with a higher tendency for specialisation in the dry season. This suggests that the mosquito population may be more susceptible to control measures in the dry season compared to the rainy season. Plastic containers are the keystone larval habitat resources that sustain high species diversity and maintain the stability of mosquito community structures against external disturbances. As such, we argue that removal of plastic containers can weaken the robustness of the mosquito community leading to species extinction.

Resistance to DDT has been established in *Aedes aegypti* populations at the study sites. Moreover, resistance to pyrethroid insecticides is emerging in the same populations, as illustrated by the presence of knockdown resistance gene V1016G mutations. Pyrethroids are the most recommended insecticides for vector control. Therefore, urgent actions must be taken to identify the origins and distribution of the observed resistance and possibly other types of *kdr* mutation polymorphisms in *Ae. aegypti* populations of the Zanzibar archipelago. Resistance management must also be implemented to safeguard the efficacy of pyrethroids for use during eventual outbreaks.

Taken together, my results provide clear evidence that effective elimination of mosquito-borne diseases in Zanzibar requires integrative efforts from the government, private investors, and the community. The need to implement a green economy to preserve human and environmental health is equally evident.

## REFERENCES

- Abe M, McCall PJ, Lenhart A, Villegas E & Kroeger A (2005) The Buen Pastor cemetery in Trujillo, Venezuela: measuring dengue vector output from a public area. *Trop Med Int Health* 10: 597-603. doi:10.1111/j.1365-3156.2005.01428.x.
- Abedi ZH & Brown AWA (1960) Development and reversion of DDT resistance in *Aedes aegypti*. *Can J Gen Cyt* 2: 252-261.
- Abilio AP, Abudasse G, Kampango A, Candrinho B, Sitoi S, Luciano J, Tembisse D, Sibindy S, de Almeida APG, Garcia GA, David MR, Maciel-de-Freitas R & Gudo ES (2018) Distribution and breeding sites of *Aedes aegypti* and *Aedes albopictus* in 32 urban/peri-urban districts of Mozambique: implication for assessing the risk of arbovirus outbreaks. *PLoS Negl Trop Dis* 12: e0006692. doi:10.1371/journal.pntd.0006692.
- Achee NL, Gould F, Perkins TA, Reiner RC, Jr., Morrison AC, Ritchie SA, Gubler DJ, Teyssou R & Scott TW (2015) A critical assessment of vector control for dengue prevention. *PLoS Negl Trop Dis* 9: e0003655. doi:10.1371/journal.pntd.0003655.
- Afify A & Galizia CG (2015) Chemosensory Cues for Mosquito Oviposition Site Selection. *J Med Entomol* 52: 120-130. doi:10.1093/jme/tju024.
- AfricaGuide (2019) Zanzibar Guide: AfricaGuide.
- Agence Nationale de Sécurité Sanitaire (2021) Résistance des moustiques vecteurs aux insecticides, p. 188.
- Agha SB, Tchouassi DP, Turell MJ, Bastos ADS & Sang R (2022) Risk assessment of urban yellow fever virus transmission in Kenya: is *Aedes aegypti* an efficient vector? *Emerging Microbes & Infections* 11: 1272-1280. doi:10.1080/22221751.2022.2063762.
- Ahmad M, Hassan V, Mohammad Ali O, Mohammad Reza A, Hamideh E, Ezatoddin J, Minoo M, Norair P & Ahmad Ali H-B (2012) Ecology of *Anopheles stephensi* in a Malarious Area, Southeast of Iran. *Acta Medica Iranica* 50: 61-65.

- Ahmad Zaki Z, Che Dom N & Ahmed Alhothily I (2020) Efficacy of *Bacillus thuringiensis* Treatment on *Aedes* Population Using Different Applications at High-Rise Buildings. *Trop Med Infect Dis* 5. doi:10.3390/tropicalmed5020067.
- Ahmed A, Khogali R, Elnour M-AB, Nakao R & Salim B (2021) Emergence of the invasive malaria vector *Anopheles stephensi* in Khartoum State, Central Sudan. *Parasit Vectors* 14: 511. doi:10.1186/s13071-021-05026-4.
- Al Nazawi AM, Aqili J, Alzahrani M, McCall PJ & Weetman D (2017) Combined target site (kdr) mutations play a primary role in highly pyrethroid resistant phenotypes of *Aedes aegypti* from Saudi Arabia. *Parasit Vectors* 10: 161. doi:10.1186/s13071-017-2096-6.
- Ali MA, James OC, Mohamed AA, Joachim A, Mubi M & Omodior O (2020) Etiologic Agents of Fever of Unknown Origin Among Patients Attending Mnazi Mmoja Hospital, Zanzibar. *Journal of Community Health* 45: 1073-1080. doi:10.1007/s10900-020-00832-w.
- Andersson N, Nava-Aguilera E, Arostegui J, Morales-Perez A, Suazo-Laguna H, Legorreta-Soberanis J, Hernandez-Alvarez C, Fernandez-Salas I, Paredes-Solis S, Balmaseda A, Cortes-Guzman AJ, Serrano de Los Santos R, Coloma J, Ledogar RJ & Harris E (2015) Evidence based community mobilization for dengue prevention in Nicaragua and Mexico (Camino Verde, the Green Way): cluster randomized controlled trial. *BMJ* 351: h3267. doi:10.1136/bmj.h3267.
- Asmare Y, Hill SR, Hopkins RJ, Tekie H & Ignell R (2017) The role of grass volatiles on oviposition site selection by *Anopheles arabiensis* and *Anopheles coluzzii*. *Malar J* 16: 65. doi:10.1186/s12936-017-1717-z.
- Awolola TS, Oduola AO, Obansa JB, Chukwurar NJ & Unyimadu JP (2007) *Anopheles gambiae* s.s. breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *J Vector Borne Dis* 44: 241-244.
- Azrag RS & Mohammed BH (2018) *Anopheles arabiensis* in Sudan: a noticeable tolerance to urban polluted larval habitats associated with resistance to Temephos. *Malar J* 17: 204. doi:10.1186/s12936-018-2350-1.

- Balabanidou V, Kefi M, Aivaliotis M, Koidou V, Girotti JR, Mijailovsky SJ, Juarez MP, Papadogiorgaki E, Chalepakis G, Kampouraki A, Nikolaou C, Ranson H & Vontas J (2019) Mosquitoes cloak their legs to resist insecticides. *Proc Biol Sci* 286: 20191091. doi:10.1098/rspb.2019.1091.
- Balkew M, Mumba P, Dengela D, Yohannes G, Getachew D, Yared S, Chibsa S, Murphy M, George K, Lopez K, Janies D, Choi SH, Spear J, Irish SR & Carter TE (2020) Geographical distribution of *Anopheles stephensi* in eastern Ethiopia. *Parasit Vectors* 13: 35. doi:10.1186/s13071-020-3904-y.
- Banerjee S, Aditya G & Saha GK (2013) Household disposables as breeding habitats of dengue vectors: Linking wastes and public health. *Waste Management* 33: 233-239.
- Bang YH, Jatanasen S & Tonn RJ (1971) Development and reversion of DDT resistance in an *Aedes aegypti* population in Bangkok, Thailand. *Bull World Health Organ* 45: 404-410.
- Barredo E & DeGennaro M (2020) Not Just from Blood: Mosquito Nutrient Acquisition from Nectar Sources. *Trends Parasitol* 36: 473-484. doi:<https://doi.org/10.1016/j.pt.2020.02.003>.
- Bascompte J (2010) Ecology. Structure and dynamics of ecological networks. *Science* 329: 765-766. doi:10.1126/science.1194255.
- Bascompte J, Jordano P & Olesen JM (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* 121: 1154—1162.
- Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C & Kaiser A (2020) Mosquitoes: Identification, Ecology and control. 3 edn. Springer, New York.
- Beer N, Ali AS, Shakely D, Elfving K, Al-Mafazy AW, Msellem M, Petzold M, Bjorkman A & Kallander K (2013) High effective coverage of vector control interventions in children after achieving low malaria transmission in Zanzibar, Tanzania. *Malar J* 12: 38. doi:10.1186/1475-2875-12-38.

- Beier JC, Keating J, Githure JJ, Macdonald MB, Impoinvil DE & Novak RJ (2008) Integrated vector management for malaria control. *Malar J* 7 Suppl 1: S4. doi:10.1186/1475-2875-7-S1-S4.
- Belkin JN (1962) *The mosquito of the South Pacific (Diptera, Culicidae)*. University of California Press.
- Benedum CM, Seidahmed OME, Eltahir EAB & Markuzon N (2018) Statistical modeling of the effect of rainfall flushing on dengue transmission in Singapore. *PLoS Negl Trop Dis* 12: e0006935. doi:10.1371/journal.pntd.0006935.
- Benelli G, Jeffries CL & Walker T (2016) Biological Control of Mosquito Vectors: Past, Present, and Future. *Insects* 7. doi:10.3390/insects7040052.
- Berger TO (1975) *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*. U. S. Department of Health, Education, and Welfare, Public Health Service.
- Berry IM, Eyase F, Pollett S, Konongoi SL, Figueroa K, Ofula V, Koka H, Koskei E, Nyunja A, Mancuso JD, Jarman RG & Sang R (2018) Recent outbreaks of chikungunya virus (CHIKV) in Africa and Asia are driven by a variant carrying mutations associated with increased fitness for *Aedes aegypti*. bioRxiv: 373316. doi:10.1101/373316.
- Berry NL, Overholt EP, Fisher TJ & Williamson CE (2020) Dissolved organic matter protects mosquito larvae from damaging solar UV radiation. *Plos one* 15: e0240261.
- Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle KE, Moyes CL, Henry A, Eckhoff PA, Wenger EA, Briet O, Penny MA, Smith TA, Bennett A, Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray CL, Smith DL, Hay SI, Cibulskis RE & Gething PW (2015) The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526: 207-211. doi:10.1038/nature15535.
- Bidlingmayer WL (1967) A comparison of trapping methods for adult mosquitoes: species response and environmental influence. *J Med Entomol* 4: 200-220.

- Bisset JA, Rodriguez MM, Piedra LA, Cruz M, Gutierrez G & Ruiz A (2020) Reversal of Resistance to the Larvicide Temephos in an *Aedes aegypti* (Diptera: Culicidae) Laboratory Strain From Cuba. *J Med Entomol* 57: 801-806. doi:10.1093/jme/tjz206.
- Bjorkman A, Shakely D, Ali AS, Morris U, Mkali H, Abbas AK, Al-Mafazy AW, Haji KA, Mcha J, Omar R, Cook J, Elfving K, Petzold M, Sachs MC, Aydin-Schmidt B, Drakeley C, Msellem M & Martensson A (2019) From high to low malaria transmission in Zanzibar-challenges and opportunities to achieve elimination. *BMC Med* 17: 14. doi:10.1186/s12916-018-1243-z.
- Blüthgen N, Menzel F & Blüthgen N (2006) Measuring specialization in species interaction networks. *BMC Ecology* 6: 9. doi:10.1186/1472-6785-6-9.
- Bolker B (2007) *Ecological model and data in R*. Princeton University Press.
- Bonizzoni M, Gasperi G, Chen X & James AA (2013) The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends Parasitol* 29: 460-468. doi:10.1016/j.pt.2013.07.003.
- Borel DT, Nana-Ndjangwo Stella M, Vasileia B, Konstantinos M, Makoudjou I, Awono-Ambene P, Charles W, Tchuinkam T, John V & Antonio-Nkondjio C (2021a) First Evidence of the Implication of Cuticular Based Mechanisms in *Aedes Aegypti* Populations Resistant To Pyrethroids and DDT in Cameroon. *Research Square*. doi:10.21203/rs.3.rs-860941/v1.
- Borel DT, Nana-Ndjangwo Stella M, Vasileia B, Konstantinos M, Makoudjou I, Awono-Ambene P, Charles W, Tchuinkam T, John V & Antonio-Nkondjio C (2021b) First Evidence of the Implication of Cuticular Based Mechanisms in *Aedes Aegypti* Populations Resistant To Pyrethroids and DDT in Cameroon. *Research Square*. doi:10.21203/rs.3.rs-860941/v1.
- Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, Ghani A, Drakeley C & Gosling R (2012) Hitting hotspots: spatial targeting of malaria for control and elimination. *PLoS Med* 9: e1001165. doi:10.1371/journal.pmed.1001165.

- Bowman LR, Donegan S & McCall PJ (2016) Is Dengue Vector Control Deficient in Effectiveness or Evidence?: Systematic Review and Meta-analysis. *PLoS Negl Trop Dis* 10: e0004551. doi:10.1371/journal.pntd.0004551.
- Brady OJ & Hay SI (2020) The Global Expansion of Dengue: How *Aedes aegypti* Mosquitoes Enabled the First Pandemic Arbovirus. *Annu Rev Entomol* 65: 191-208. doi:10.1146/annurev-ento-011019-024918.
- Briegel H & Timmermann SE (2001) *Aedes albopictus* (Diptera: Culicidae): Physiological Aspects of Development and Reproduction. *J Med Entomol* 38: 566-571. doi:10.1603/0022-2585-38.4.566.
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M & Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal* 9: 378–400.
- Brown ZS, Dickinson KL & Kramer RA (2013) Insecticide resistance and malaria vector control: the importance of fitness cost mechanisms in determining economically optimal control trajectories. *J Econ Entomol* 106: 366-374. doi:10.1603/ec11365.
- Buhler C, Winkler V, Runge-Ranzinger S, Boyce R & Horstick O (2019) Environmental methods for dengue vector control - A systematic review and meta-analysis. *PLoS Negl Trop Dis* 13: e0007420. doi:10.1371/journal.pntd.0007420.
- Burgos E, Ceva H, Perazzo RPJ, Devoto M, Medan D, Zimmermann M & Delbue AM (2007) Why nestdness in mutualistic networks? *J Theor Biology* 249: 307 - 313.
- Burkot T & Ichimori K (2002) The PacELF programme: will mass drug administration be enough? *Trends Parasitol* 18: 109-115. doi:10.1016/s1471-4922(01)02221-8.
- Burkot TR (1988) Non-random host selection by anopheline mosquitoes. *Parasitol Today* 4: 156-162.
- Burkot TR, Handzel T, Schmaedick MA, Tufa J, Roberts JM & Graves PM (2007) Productivity of natural and artificial containers for *Aedes polynesiensis* and *Aedes aegypti* in four American Samoan villages. *Med Vet Entomol* 21: 22-29. doi:10.1111/j.1365-2915.2007.00667.x.

- Caldwell JM, LaBeaud AD, Lambin EF, Stewart-Ibarra AM, Ndenga BA, Mutuku FM, Krystosik AR, Ayala EB, Anyamba A, Borbor-Cordova MJ, Damoah R, Grossi-Soyster EN, Heras FH, Ngugi HN, Ryan SJ, Shah MM, Sippy R & Mordecai EA (2021) Climate predicts geographic and temporal variation in mosquito-borne disease dynamics on two continents. *Nat Commun* 12: 1233. doi:10.1038/s41467-021-21496-7.
- Campbell-Lendrum D, Manga L, Bagayoko M & Sommerfeld J (2015) Climate change and vector-borne diseases: what are the implications for public health research and policy? *Philos Trans R Soc Lond B Biol Sci* 370. doi:10.1098/rstb.2013.0552.
- Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, Marfin AA, Solomon T, Tsai TF, Tsu VD & Ginsburg AS (2011) Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* 89: 766-774E. doi:10.2471/BLT.10.085233.
- Cappellari A & Marini L (2021) Improving insect conservation across heterogeneous landscapes using species-habitat networks. *PeerJ* 9: e10563. doi:10.7717/peerj.10563.
- CaraDonna PJ, Petry WK, Brennan RM, Cunningham JL, Bronstein JL, Waser NM & Sanders NJ (2017) Interaction rewiring and the rapid turnover of plant–pollinator networks. *Ecol. Lett.* 20: 385–394.
- Castro MC, Kanamori S, Kannady K, Mkude S, Killeen GF & Fillinger U (2010) The importance of drains for the larval development of lymphatic filariasis and malaria vectors in Dar es Salaam, United Republic of Tanzania. *PLoS Negl Trop Dis* 4: e693. doi:10.1371/journal.pntd.0000693.
- Castro MC, Tsuruta A, Kanamori S, Kannady K & Mkude S (2009) Community-based environmental management for malaria control: evidence from a small-scale intervention in Dar es Salaam, Tanzania. *Malar J* 8.
- Cattarino L, Rodriguez-Barraquer I, Imai N, Cummings DAT & Ferguson NM (2020) Mapping global variation in dengue transmission intensity. *Sci Transl Med* 12.
- Chang K, Chen CD, Shih CM, Lee TC, Wu MT, Wu DC, Chen YH, Hung CH, Wu MC, Huang CC, Lee CH & Ho CK (2016) Time-Lagging Interplay Effect and Excess Risk of

- Meteorological/Mosquito Parameters and Petrochemical Gas Explosion on Dengue Incidence. *Sci Rep* 6: 35028. doi:10.1038/srep35028.
- Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK & Ellison AM (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Eco Monograph* 84: 45-67.
- Charlwood JD (1994) The control of *Culex quinquefasciatus* breeding in septic tanks using expanded polystyrene beads in southern Tanzania. *Trans R Soc Trop Med Hyg* 88: 380.
- Charlwood JD & Braganca M (2012) The effect of rainstorms on adult *Anopheles funestus* behavior and survival. *J Vector Ecol* 37: 252-256. doi:10.1111/j.1948-7134.2012.00224.x.
- Charlwood JD, Pinto J, Ferrara PR, Sousa CA, Ferreira C, Gil V & Do Rosario VE (2003) Raised houses reduce mosquito bites. *Malar J* 2: 45. doi:10.1186/1475-2875-2-45.
- Chathuranga WGD, Karunaratne SHPP, Fernando BR & De Silva WAPP (2018) Diversity, distribution, abundance, and feeding pattern of tropical ornithophilic mosquitoes. *Journal of Vector Ecology* 43: 158-167. doi:<https://doi.org/10.1111/jvec.12295>.
- Chaves LF, Harrington LC, Keogh CL, Nguyen AM & Kitron UD (2010) Blood feeding patterns of mosquitoes: random or structured? *Front Zool* 7: 3. doi:10.1186/1742-9994-7-3.
- Chipwaza B, Sumaye RD, Weisser M, Gingo W, Yeo NK-W, Amrun SN, Okumu FO & Ng LFP (2021a) Occurrence of 4 Dengue Virus Serotypes and Chikungunya Virus in Kilombero Valley, Tanzania, During the Dengue Outbreak in 2018. *Open Forum Infectious Diseases* 8. doi:10.1093/ofid/ofaa626.
- Chipwaza B, Sumaye RD, Weisser M, Gingo W, Yeo NK, Amrun SN, Okumu FO & Ng LFP (2021b) Occurrence of 4 Dengue Virus Serotypes and Chikungunya Virus in Kilombero Valley, Tanzania, During the Dengue Outbreak in 2018. *Open Forum Infect Dis* 8: ofaa626. doi:10.1093/ofid/ofaa626.
- Christie J (1872) Remarks on "Kidinga Pepo": A Peculiar Form of Exanthematous Disease. *Br Med J* 1: 577-579. doi:10.1136/bmj.1.596.577.

- Christophers R (1960) *Aedes Aegypti*(Linnaeus), the Yellow Fever Mosquito. Cambridge University Press, New York.
- Ciota AT (2017) West Nile virus and its vectors. *Curr Opin Insect Sci* 22: 28-36. doi:10.1016/j.cois.2017.05.002.
- Coetzee M (2020) Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malar J* 19.
- Cohuet A, Simard F, Wondji CS, Antonio-Nkondjio C, Awono-Ambene P & D. F (2004) High malaria transmission intensity due to *Anopheles funestus* (Diptera: Culicidae) in a village of savannah-forest transition area in Cameroon. *J Med Entomol* 41: 901-905.
- Coleman PG & Alphey L (2004) Editorial: Genetic control of vector populations: an imminent prospect. *Tropical Medicine & International Health* 9: 433-437.
- Colón-González FJ, Sewe MO, Tompkins AM, Sjödin H, Casallas A, Rocklöv J, Caminade C & Lowe R (2021) Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. *The Lancet Planetary Health* 5: e404-e414. doi:10.1016/S2542-5196(21)00132-7.
- Colwell RK & Coddington JA (1994) Estimating Terrestrial Biodiversity through Extrapolation. *Philos Trans R Soc Lond B Biol Sci* 345: 101-118.
- Conteh L, Sicuri E, Manzi F, Hutton G, Obonyo B, Tediosi F, Biao P, Masika P, Matovu F, Otieno P, Gosling RD, Hamel M, Odhiambo FO, Grobusch MP, Kremsner PG, Chandramohan D, Aponte JJ, Egan A, Schellenberg D, Macete E, Slutsker L, Newman RD, Alonso P, Menendez C & Tanner M (2010) The cost-effectiveness of intermittent preventive treatment for malaria in infants in Sub-Saharan Africa. *Plos one* 5: e10313. doi:10.1371/journal.pone.0010313.
- Coosemans M & Carnevale P (1995) Malaria vector control: a critical review on chemical methods and insecticides. *Ann Soc Belg Med Trop* 75: 13-31.
- Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbeto M, Hougaard JM & Rowland M (2007) Multiple insecticide resistance mechanisms in

- Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Trop* 101: 207-216. doi:10.1016/j.actatropica.2007.01.005.
- Cornel AJ & Hunt RH (1991) *Aedes albopictus* in Africa? First records of live specimens in imported tires in Cape Town. *J Am Mosq Control Assoc* 7: 107-108.
- Couret J, Dotson E & Benedict MQ (2014) Temperature, Larval Diet, and Density Effects on Development Rate and Survival of *Aedes aegypti* (Diptera: Culicidae). *Plos one* 9: e87468. doi:10.1371/journal.pone.0087468.
- Cugola FR, Fernandes IR, Russo FB, Freitas BC, Dias JL, Guimaraes KP, Benazzato C, Almeida N, Pignatari GC, Romero S, Polonio CM, Cunha I, Freitas CL, Brandao WN, Rossato C, Andrade DG, Faria Dde P, Garcez AT, Buchpigel CA, Braconi CT, Mendes E, Sall AA, Zanotto PM, Peron JP, Muotri AR & Beltrao-Braga PC (2016) The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* 534: 267-271. doi:10.1038/nature18296.
- Cunha M, Duarte-Neto AN, Pour SZ, Ortiz-Baez AS, Černý J, Pereira B, Braconi CT, Ho YL, Perondi B, Sztajnbock J, Alves V, Dolhnikoff M, Holmes EC, Saldiva P & Zanotto P (2019) Origin of the São Paulo Yellow Fever epidemic of 2017-2018 revealed through molecular epidemiological analysis of fatal cases. *Sci Rep* 9: 20418.
- Curtis CF, Cook LM & Wood RJ (1978) Selection for and against insecticide resistance and possible methods of inhibiting the evolution of resistance in mosquitoes. *Ecological Entomology* 3: 273-287. doi:<https://doi.org/10.1111/j.1365-2311.1978.tb00928.x>.
- Curtis CF & Townson H (1998) Malaria: existing methods of vector control and molecular entomology. *British Medical Bulletin* 54: 311-325.
- Dambach P, Winkler V, Bärnighausen T, Traoré I, Ouedraogo S, Sié A, Sauerborn R, Becker N & Louis VR (2020) Biological larviciding against malaria vector mosquitoes with *Bacillus thuringiensis israelensis* (Bti) – Long term observations and assessment of repeatability during an additional intervention year of a large-scale field trial in rural Burkina Faso. *Global Health Action* 13: 1829828.

- Dao A, Yaro AS, Diallo M, Timbiné S, Huestis DL, Kassogué Y, Traoré AI, Sanogo ZL, Samaké D & Lehmann T (2014) Signatures of aestivation and migration in Sahelian malaria mosquito populations. *Nature* 516: 387-390. doi:10.1038/nature13987.
- De Meillon B (1951) Species and varieties of malaria vectors in Africa and their bionomics. *Bull World Health Organ* 4: 419-441.
- De Silva PM & Marshall JM (2012) Factors contributing to urban malaria transmission in sub-saharan Africa: a systematic review. *J Trop Med* 2012: 819563. doi:10.1155/2012/819563.
- de Silva WAPP, Bernal XE, Chathuranga WGD, Herath BP, Ekanayake C, Abeysundara HTK & Karunaratne SHPP (2020) Feeding patterns revealed host partitioning in a community of frog-biting mosquitoes. *Ecological Entomology* 45: 988-996. doi:<https://doi.org/10.1111/een.12874>.
- Debboun M, Frances SP & Strickman DA (2015) *Insect Repellents Handbook*. 2 edn. CRC Press.
- Degefa T, Yewhalaw D, Zhou G, Lee M-c, Atieli H, Githeko AK & Yan G (2017) Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission. *Malar J* 16: 443. doi:10.1186/s12936-017-2098-z.
- Deisting F & Rey S (2015) Determinants of Tourism In French overseas Departments And Collectivities. *Région et Développement* 42: 215-230.
- Dieme C, Bechah Y, Socolovschi C, Audoly G, Berenger J-M, Faye O, Raoult D & Parola P (2015) Transmission potential of *Rickettsia felis* infection by *Anopheles gambiae* mosquitoes. *Proceedings of the National Academy of Sciences* 112: 8088. doi:10.1073/pnas.1413835112.
- Dinglasan RR, Devenport M, Florens L, Johnson JR, McHugh CA, Donnelly-Doman M, Carucci DJ, Yates JR, 3rd & Jacobs-Lorena M (2009) The *Anopheles gambiae* adult midgut peritrophic matrix proteome. *Insect Biochem Mol Biol* 39: 125-134. doi:10.1016/j.ibmb.2008.10.010.

- Diniz DF, de Melo-Santos MA, Santos EM, Beserra EB, Helvecio E, de Carvalho-Leandro D, dos Santos BS, de Menezes Lima VL & Ayres CF (2015) Fitness cost in field and laboratory *Aedes aegypti* populations associated with resistance to the insecticide temephos. *Parasit Vectors* 8: 662. doi:10.1186/s13071-015-1276-5.
- Djiappi-Tchamen B, Nana-Ndjangwo MS, Mavridis K, Talipouo A, Nchoutpouen E, Makoudjou I, Bamou R, Mayi AM, Awono-Ambene P, Tchuinkam T, Vontas J & Antonio-Nkondjio C (2021) Analyses of Insecticide Resistance Genes in *Aedes aegypti* and *Aedes albopictus* Mosquito Populations from Cameroon. *Genes* 12. doi:10.3390/genes12060828.
- Dormann CF, Frund J, Bluthgen N & Gruber B (2009) Indices, graphs and null models: Analyzing bipartite ecological networks. *Open Ecol J* 2: 7-24.
- Du Y, Nomura Y, Zhorov BS & Dong K (2016) Sodium Channel Mutations and Pyrethroid Resistance in *Aedes aegypti*. *Insects* 7. doi:10.3390/insects7040060.
- Duman-Scheel M, Eggleston KK, Achee NL, Grieco JP & Hapairai LK (2018) Mosquito control practices and perceptions: An analysis of economic stakeholders during the Zika epidemic in Belize, Central America. *Plos one* 13: e0201075. doi:10.1371/journal.pone.0201075.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797. doi:10.1093/nar/gkh340.
- Edwards FH (1941) Mosquitoes of the Ethiopian Region. III. Culicine Adults and Pupae. British Museum (Natural History), London, United Kingdom.
- EHL Insights (2020) Going Plastic-Free: Hotels and Airlines Reducing Plastic Use, Vol. 2022.
- Eldridge BF (2005) Mosquito, the Culicidae: Biology of Disease Vectors (ed. by WC Marquardt) Elsevier Academic Press, pp. 95-111.
- Ellis BR & Wilcox BA (2009) The ecological dimensions of vector-borne disease research and control. *Cad Saude Publica* 25 Suppl 1: S155-167. doi:10.1590/s0102-311x2009001300015.

- Eritja R, Palmer JRB, Roiz D, Sanpera-Calbet I & Bartumeus F (2017) Direct Evidence of Adult *Aedes albopictus* Dispersal by Car. *Sci Rep* 7: 14399. doi:10.1038/s41598-017-12652-5.
- Escobar A & Južnič-Zonta Ž (2022) Mosquito Alert Dataset. Version 1.11: by CREAMF) [www.GBIF.org](http://www.GBIF.org).
- Fan Y, O'Grady P, Yoshimizu M, Ponlawat A, Kaufman PE & Scott JG (2020) Evidence for both sequential mutations and recombination in the evolution of *kdr* alleles in *Aedes aegypti*. *PLoS Negl Trop Dis* 14: e0008154. doi:10.1371/journal.pntd.0008154.
- Faulde MK, Rueda LM & Khairah BA (2014) First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. *Acta Tropica* 139: 39-43. doi:<https://doi.org/10.1016/j.actatropica.2014.06.016>.
- Ferguson HM, Dornhaus A, Beeche A, Borgemeister C, Gottlieb M, Mulla MS, Gimnig JE, Fish D & Killeen GF (2010) Ecology: a prerequisite for malaria elimination and eradication. *PLoS Med* 7: e1000303-e1000303. doi:10.1371/journal.pmed.1000303.
- Ferguson NM (2018) Challenges and opportunities in controlling mosquito-borne infections. *Nature* 559: 490-497. doi:10.1038/s41586-018-0318-5.
- Ferraguti M, Martinez-de la Puente J, Roiz D, Ruiz S, Soriguer R & Figuerola J (2016) Effects of landscape anthropization on mosquito community composition and abundance. *Sci Rep* 6: 29002. doi:10.1038/srep29002.
- Fikrig K & Harrington LC (2021) Understanding and interpreting mosquito blood feeding studies: the case of *Aedes albopictus*. *Trends Parasitol* 37: 959-975. doi:<https://doi.org/10.1016/j.pt.2021.07.013>.
- Finda MF, Moshi IR, Monroe A, Limwagu AJ, Nyoni AP, Swai JK, Ngowo HS, Minja EG, Toe LP, Kaindoa EW, Coetzee M, Manderson L & Okumu FO (2019) Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. *Plos one* 14: e0217414-e0217414. doi:10.1371/journal.pone.0217414.

- Flaibani N, Pérez AA, Barbero IM & Burroni NE (2020) Different approaches to characterize artificial breeding sites of *Aedes aegypti* using generalized linear mixed models. *Infectious Diseases of Poverty* 9: 107. doi:10.1186/s40249-020-00705-3.
- Flores HA & O'Neill SL (2018) Controlling vector-borne diseases by releasing modified mosquitoes. *Nat Rev Microbiol* 16: 508-518. doi:10.1038/s41579-018-0025-0.
- Flores HA & O'Neill SL (2018) Controlling vector-borne diseases by releasing modified mosquitoes. *Nature Reviews Microbiology* 16: 508-518. doi:10.1038/s41579-018-0025-0.
- Fontenille D & Lochouart L (1999) The complexity of the malaria vectorial system in Africa. *Parassitologia* 41: 267-271.
- Fontenille D & Toto JC (2001) *Aedes* (*Stegomyia*) *albopictus* (Skuse), a potential new Dengue vector in southern Cameroon. *Emerg Infect Dis* 7: 1066-1067. doi:10.3201/eid0706.010631.
- Forum Z (2019a) Climate & Soils: Zanzinet.
- Forum Z (2019b) Economy: Zanzinet.
- Foster WA & Takken W (2004) Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. *Bull Entomol Res* 94: 145-157.
- Foster WA & Walker ED (2019) *Mosquitoes: Culicidae: Med Vet Entomol* (ed. by GR Mullen & LC Durden) Elsevier.
- Fouet C & Kamdem C (2019) Integrated Mosquito Management: Is Precision Control a Luxury or Necessity? *Trends Parasitol* 35: 85-95. doi:10.1016/j.pt.2018.10.004.
- Francis S, Frank C, Buchanan L, Green S, Stennett-Brown R, Gordon-Strachan G, Rubio-Palis Y, Grant C, Alexander-Lindo RL, Nwokocha C, Robinson D & Delgoda R (2021) Challenges in the control of neglected insect vector diseases of human importance in the Anglo-Caribbean. *One Health* 13: 100316. doi:10.1016/j.onehlt.2021.100316.

- Franklin DC & Whelan PI (2009) Tropical mosquito assemblages demonstrate 'textbook' annual cycles. *Plos one* 4: e8296. doi:10.1371/journal.pone.0008296.
- Franklinos LHV, Jones KE, Redding DW & Abubakar I (2019) The effect of global change on mosquito-borne disease. *Lancet Infect Dis* 19: e302-e312. doi:10.1016/S1473-3099(19)30161-6.
- Fraser KJ, Mwandigha L, Traore SF, Traore MM, Doumbia S, Junnila A, Revay E, Beier JC, Marshall JM, Ghani AC & Müller G (2021) Estimating the potential impact of Attractive Targeted Sugar Baits (ATSBs) as a new vector control tool for *Plasmodium falciparum* malaria. *Malar J* 20: 151. doi:10.1186/s12936-021-03684-4.
- Freeman JC, San Miguel K & Scott JG (2021) All resistance alleles are not equal: the high fitness cost of super-kdr in the absence of insecticide. *Pest Manag Sci* 77: 3693-3697. doi:10.1002/ps.6115.
- Gardner AM, Allan BF, De Urioste-Stone S, Lieberthal BA, Mackay A, Soliman A & Wang S (2019) Feedbacks Between Mosquito-Borne Disease Transmission and Tourism in the Americas: Ecological Society of America.
- Garrett-Jones C (1950) A dispersion of mosquitoes by wind. *Nature* 165: 285.
- Garrett-Jones C (1956) Migratory flight in anopheline mosquitoes in the Middle East. *Bull Endem Dis (Baghdad)* 2: 79-87.
- Garrett-Jones C (1962) The possibility of active long-distance migrations by *Anopheles pharoensis* Theobald. *Bull World Health Organ* 27: 299-302.
- Gary Jr RE & Foster WA (2006) Diel timing and frequency of sugar feeding in the mosquito *Anopheles gambiae*, depending on sex, gonotrophic state and resource availability. *Med Vet Entomol* 20: 308-316. doi:<https://doi.org/10.1111/j.1365-2915.2006.00638.x>.
- Gary RE, Jr. & Foster WA (2001) Effects of Available Sugar on the Reproductive Fitness and Vectorial Capacity of the Malaria Vector *Anopheles gambiae* (Diptera: Culicidae). *J Med Entomol* 38: 22-28. doi:10.1603/0022-2585-38.1.22.
- Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HC, Gould F, Hastings I, Marshall J, Ranson H, Rowland M, Shaman J & Lindsay SW (2013) The importance

- of mosquito behavioural adaptations to malaria control in Africa. *Evolution* 67: 1218-1230. doi:10.1111/evo.12063.
- Gautret P, Simon F, Hervius Askling H, Bouchaud O, Leparç-Goffart I, Ninove L, Parola P & EuroTravNet (2010) Dengue type 3 virus infections in European travellers returning from the Comoros and Zanzibar, February-April 2010. *Euro Surveill* 15: 19541.
- Gaythorpe KA, Hamlet A, Cibrelus L, Garske T & Ferguson NM (2020) The effect of climate change on yellow fever disease burden in Africa. *Elife* 9: e55619. doi:10.7554/eLife.55619.
- Gaythorpe KAM, Hamlet A, Jean K, Garkauskas Ramos D, Cibrelus L, Garske T & Ferguson N (2021) The global burden of yellow fever. *Elife* 10: e64670. doi:10.7554/eLife.64670.
- Geissbuhler Y, Kannady K, Chaki PP, Emidi B, Govella NJ, Mayagaya V, Kiama M, Mtasiwa D, Mshinda H, Lindsay SW, Tanner M, Fillinger U, de Castro MC & Killeen GF (2009) Microbial larvicide application by a large-scale, community-based program reduces malaria infection prevalence in urban Dar es Salaam, Tanzania. *Plos one* 4: e5107. doi:10.1371/journal.pone.0005107.
- Georghiou GP (1990) Overview of Insecticide Resistance, Vol. 421: Managing Resistance to Agrochemicals (ed. by MB Green, HM LeBaron & WK Morberg) American Chemical Society, pp. 18-41.
- Georghiou GP (1994) Principles of insecticide resistance management. *Phytoprotection* 75: 51-59.
- Georghiou GP & Taylor CE (1977) Genetic and biological influences in the evolution of insecticide resistance. *J Econ Entomol* 70: 319-323. doi:10.1093/jee/70.3.319.
- Gillies MT (1956) The problem of exophily in *Anopheles gambiae*. *Bull World Health Organ* 15: 437-449.
- Gillies MT (1961) Studies on the dispersion and survival of *Anopheles gambiae* Giles in East Africa, by means of marking and release experiments. *Bull Entomol Res* 52: 99-127.

- Gillies MT & De Meillon B (1968) The Anophelinae of the Africa south of the sahara (Ethiopian zoographical region). South African Institute for Medical Research, South Africa.
- Gilroy AB & Chwatt LJ (1945) Mosquito-control by swamp drainage in the costal bel of Nigeria. *Ann Trop Med Parasitol* 39: 20-40.
- Goodyer LI, Croft AM, Frances SP, Hill N, Moore SJ, Onyango SP & Debboun M (2010) Expert Review of the Evidence Base for Arthropod Bite Avoidance. *Journal of Travel Medicine* 17: 182-192. doi:10.1111/j.1708-8305.2010.00402.x.
- Gouagna L-C, Poueme RS, Roch Dabiré K, Ouédraogo J-B, Fontenille D & Simard F (2010) Patterns of Sugar Feeding and Host Plant Preferences in Adult Males of *An. gambiae* (Diptera: Culicidae). *Journal of Vector Ecology* 35: 267-276, 210.
- Green P & MacLeod CJ (2016) SIMR: an R package for power analysis of generalized linear mixed models by simulation. *Methods Ecology and Evolution* 7: 493–498.
- Grobbelaar AA, Weyer J, Moolla N, Jansen van Vuren P, Moises F & Paweska JT (2016) Resurgence of Yellow Fever in Angola, 2015-2016. *Emerg Infect Dis* 22: 1854-1855. doi:10.3201/eid2210.160818.
- Grossman MK, Uc-Puc V, Rodriguez J, Cutler DJ, Morran LT, Manrique-Saide P & Vazquez-Prokopec GM (2018) Restoration of pyrethroid susceptibility in a highly resistant *Aedes aegypti* population. *Biol Lett* 14. doi:10.1098/rsbl.2018.0022.
- Guindo-Coulibaly N, Adja AM, Coulibaly JT, Kpan MDS, Adou KA & Zoh DD (2019) Expansion of *Aedes africanus* (Diptera: Culicidae), a sylvatic vector of arboviruses, into an urban environment of Abidjan, Côte d'Ivoire. *Journal of Vector Ecology* 44: 248-255. doi:<https://doi.org/10.1111/jvec.12356>.
- Gulland A (2016) Zika virus is a global public health emergency, declares WHO. *BMJ* 352: i657. doi:10.1136/bmj.i657.
- Haddow AJ (1954a) Studies on the biting-habits of African mosquitoes: an appraisal of methods employed, with special reference to the twenty-four-hour catch. *Bull Entomol Res* 45: 199-242.

- Haddow AJ (1954b) Studies on the biting-habits of African mosquitoes: an appraisal of methods employed, with special reference to the twenty-four-hour catch. *Bull Ent Res* 45: 199-242.
- Haddow AJ (1956) Observations on the Biting-habits of African Mosquitos in the Genus *Eretmapodites* Theobald. *Bull Entomol Res* 46: 761-772. doi:10.1017/S0007485300037020.
- Haddow AJ (1964) The mosquitoes of Bwamba county, Uganda. II- Biting activity with special reference to the influence of micriclimate. *Bull Ent Res* 36: 33-73.
- Hahn MB, Gangnon RE, Barcellos C, Asner GP & Patz JA (2014) Influence of deforestation, logging, and fire on malaria in the Brazilian Amazon. *Plos one* 9: e85725. doi:10.1371/journal.pone.0085725.
- Haji KA, Khatib BO, Smith S, Ali AS, Devine GJ, Coetzee M & Majambere S (2013) Challenges for malaria elimination in Zanzibar: pyrethroid resistance in malaria vectors and poor performance of long-lasting insecticide nets. *Parasit Vectors* 6: 82. doi:10.1186/1756-3305-6-82.
- Haji KA, Thawer NG, Khatib BO, Mcha JH, Rashid A, Ali AS, Jones C, Bagi J, Magesa SM, Ramsan MM, Garimo I, Greer G, Reithinger R & Ngondi JM (2015) Efficacy, persistence and vector susceptibility to pirimiphos-methyl (Actellic® 300CS) insecticide for indoor residual spraying in Zanzibar. *Parasit Vectors* 8: 628. doi:10.1186/s13071-015-1239-x.
- Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
- Halstead SB (2015) Reappearance of Chikungunya, Formerly Called Dengue, in the Americas. *Emerg Infect Dis.* 21(4):557-561. 21.
- Hamer GL, Anderson TK, Donovan DJ, Brawn JD, Krebs BL, Gardner AM, Ruiz MO, Brown WM, Kitron UD, Newman CM, Goldberg TL & Walker ED (2014) Dispersal of adult culex mosquitoes in an urban west nile virus hotspot: a mark-capture study incorporating stable isotope enrichment of natural larval habitats. *PLoS Negl Trop Dis* 8: e2768-e2768. doi:10.1371/journal.pntd.0002768.

- Hanai D, Hardstone Yoshimizu M & Scott JG (2018) The Insecticide Resistance Allele *kdr*-his has a Fitness Cost in the Absence of Insecticide Exposure. *J Econ Entomol* 111: 2992-2995. doi:10.1093/jee/toy300.
- Harbach RE (2007) The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* 1668: 591 - 638.
- Harbach RE (2013) Mosquito Taxonomic Inventory, Vol. 2022: Nature History Museum (of London).
- Harbach RE (2021) Mosquito Taxonomic Inventory, Vol. 2016: Nature History Museum (of London).
- Hardy A, Mageni Z, Dongus S, Killeen G, Macklin MG, Majambare S, Ali A, Msellem M, Al-Mafazy AW, Smith M & Thomas C (2015) Mapping hotspots of malaria transmission from pre-existing hydrology, geology and geomorphology data in the pre-elimination context of Zanzibar, United Republic of Tanzania. *Parasit Vectors* 8: 41. doi:10.1186/s13071-015-0652-5.
- Harrington LC, Edman JD & Scott TW (2001) Why Do Female *Aedes aegypti* (Diptera: Culicidae) Feed Preferentially and Frequently on Human Blood? *J Med Entomol* 38: 411-422. doi:10.1603/0022-2585-38.3.411.
- Harris P, Riordan DF & Cooke D (1969) Mosquitoes Feeding on Insect Larvae. *Science* 164: 184-185. doi:10.1126/science.164.3876.184.
- Hartig F (2020) DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. R package version 0.3.3.0. <https://CRAN.R-project.org/package=DHARMA>.
- Hawley WA (1988) The biology of *Aedes albopictus*. *J Am Mosq Control Assoc Suppl* 1: 1-39.
- Helinski MEH, Hassan MaM, El-Motasim WM, Malcolm CA, Knols BGJ & El-Sayed B (2008) Towards a sterile insect technique field release of *Anopheles arabiensis* mosquitoes in Sudan: Irradiation, transportation, and field cage experimentation. *Malar J* 7: 65. doi:10.1186/1475-2875-7-65.

- Hemingway J (2005) *Biological Control of Mosquitoes: Biology of Disease Vectors* (ed. by WC Marquardt) Elsevier, Amsterdam, pp. 649-660.
- Hemingway J & Ranson H (2000) Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol* 45: 371-391. doi:10.1146/annurev.ento.45.1.371.
- Hemingway J & Ranson H (2005) Chemical control of vectors and mechanisms of resistance: *Biology of Disease Vectors* (ed. by WC Marquardt) Elsevier, Amsterdam, pp. 627-637.
- Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, Coetzee M, Simard F, Roch DK, Hinzoumbe CK, Pickett J, Schellenberg D, Gething P, Hoppe M & Hamon N (2016) Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet* 387: 1785-1788. doi:10.1016/S0140-6736(15)00417-1.
- Hilbe JM (2011) *Negative Binomial Regression*. Second edn. Cambridge University Press, Cambridge, UK.
- Hilbe JM (2014) *Problems with Zeros: Modeling Count Data* (ed. by JM Hilbe) Cambridge University Press, Cambridge, pp. 172-209.
- Hocking B (1971) Blood-sucking behaviour of terrestrial arthropods. *Ann Rev Ent* 16: 1-26.
- Hopkins GHE (1952) *Mosquito of the Ethiopian region: I. Larval bionomics of mosquitoes and taxonomy of culicine larvae*. Adlard and Son, Ltd, London, United Kingdom.
- Hribar L (2014) Mosquitoes feeding on caterpillars of the Common Buckeye butterfly, *Junonia coenia* (Lepidoptera: Nymphalidae). *The Journal of Research on the Lepidoptera* 47: 45-48.
- Hsieh TC, Ma KH & Chao A (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol and Evol* 7: 1451–1456.
- Huang Y-M (1972) *Contributions to the Mosquito Fauna of Southeast Asia. XIV. The Subgenus Stegomyia of Aedes in Southeast Asia I - The Scutellaris Group of Species*. Contribution of the American Entomological Institute: 1-111.

- Huang Y-M (1979) The Subgenus *Stegomyia* of *Aedes* in the Oriental Region with Keys to the Species (Diptera: Culicidae). Contributions of the American Entomological Institute 15: 1-83.
- Huang Y-M (1997) The Subgenus *Stegomyia* of *Aedes* in the Afrotropical Region. II. The *Dendrophilus* group of species (Diptera: Culicidae). Contributions of the American Entomological Institute 29: 1-104.
- Huang Y-M (2004) The subgenus *Stegomyia* of *Aedes* in the Afrotropical Region with keys to the species (Diptera: Culicidae). Zootaxa 700: 1-120.
- Huang YM (1986) *Aedes* (*Stegomyia*) *bromeliae* (Diptera: Culicidae), the yellow fever virus vector in East Africa. J Med Entomol 23: 196-200. doi:10.1093/jmedent/23.2.196.
- Huestis DL, Dao A, Diallo M, Sanogo ZL, Samake D, Yaro AS, Ousman Y, Linton Y-M, Krishna A, Veru L, Krajacich BJ, Faiman R, Florio J, Chapman JW, Reynolds DR, Weetman D, Mitchell R, Donnelly MJ, Talamas E, Chamorro L, Strobach E & Lehmann T (2019) Windborne long-distance migration of malaria mosquitoes in the Sahel. Nature 574: 404-408. doi:10.1038/s41586-019-1622-4.
- Hustedt JC, Doum D, Keo V, Ly S, Sam B, Chan V, Alexander N, Bradley J, Liverani M, Prasetyo DB, Rachmat A, Shafique M, Lopes S, Rithea L & Hii J (2021) Field Efficacy of Larvivorous Fish and Pyriproxyfen Combined with Community Engagement on Dengue Vectors in Cambodia: A Randomized Controlled Trial. Am J Trop Med Hyg 105: 1265-1276. doi:10.4269/ajtmh.20-1088.
- Hutson AD & Reid ME (2004) The utility of partial cross-over designs in early phase randomized prevention trials. Control Clin Trials 25: 493-501. doi:10.1016/j.cct.2004.07.005.
- Ingelbeen B, Weregemere NA, Noel H, Tshapenda GP, Mossoko M, Nsio J, Ronsse A, Ahuka-Mundeke S, Cohuet S & Kebela BI (2018) Urban yellow fever outbreak-Democratic Republic of the Congo, 2016: Towards more rapid case detection. PLoS Negl Trop Dis 12: e0007029-e0007029. doi:10.1371/journal.pntd.0007029.
- IRAC (2011) Prevention and management of insecticide resistance in vectors of public health importance. 2 edn.

- Irish SR, Kyalo D, Snow RW & Coetzee M (2020) Updated list of *Anopheles* species (Diptera: Culicidae) by country in the Afrotropical Region and associated islands. *Zootaxa* 4747: zootaxa 4747 4743 4741. doi:10.11646/zootaxa.4747.3.1.
- Ishtiaq F, Swain S & Kumar SS (2021a) *Anopheles stephensi* (Asian Malaria Mosquito). *Trends Parasitol* 37: 571-572. doi:10.1016/j.pt.2021.03.009.
- Ishtiaq F, Swain S & Kumar SS (2021b) *Anopheles stephensi* (Asian Malaria Mosquito). *Trends Parasitol* 37: 571-572. doi:10.1016/j.pt.2021.03.009.
- Jamison A, Tuttle E, Jensen R, Bierly G & Gonser R (2015) Spatial ecology, landscapes, and the geography of vector-borne disease: A multi-disciplinary review. *Applied Geography* 63: 418-426. doi:<https://doi.org/10.1016/j.apgeog.2015.08.001>.
- Johnson PC, Barry SJ, Ferguson HM & Muller P (2015) Power analysis for generalized linear mixed models in ecology and evolution. *Methods Ecol Evol* 6: 133-142. doi:10.1111/2041-210X.12306.
- Jones CM, Haji KA, Khatib BO, Bagi J, Mcha J, Devine GJ, Daley M, Kabula B, Ali AS, Majambere S & Ranson H (2013) The dynamics of pyrethroid resistance in *Anopheles arabiensis* from Zanzibar and an assessment of the underlying genetic basis. *Parasit Vectors* 6: 343. doi:10.1186/1756-3305-6-343.
- Jones CM, Machin C, Mohammed K, Majambere S, Ali AS, Khatib BO, McHa J, Ranson H & Kelly-Hope LA (2012) Insecticide resistance in *Culex quinquefasciatus* from Zanzibar: implications for vector control programmes. *Parasit Vectors* 5: 78. doi:10.1186/1756-3305-5-78.
- Juliano SA (2008) Species Interactions Among Larval Mosquitoes: Context Dependence Across Habitat Gradients. *Annu Rev Entomol* 54: 37-56. doi:10.1146/annurev.ento.54.110807.090611.
- Jupp PG (1986) *Mosquitoes of Southern Africa*. Ekogilde Publishers, Pretoria, South Africa.
- Jurat-Fuentes JL, Heckel DG & Ferre J (2021) Mechanisms of Resistance to Insecticidal Proteins from *Bacillus thuringiensis*. *Annu Rev Entomol* 66: 121-140. doi:10.1146/annurev-ento-052620-073348.

- Juri MJD, Estallo E, Almirón W, Santana M, Sartor P, Lamfri M & Zaidenberg M (2015) Satellite-derived NDVI, LST, and climatic factors driving the distribution and abundance of *Anopheles* mosquitoes in a former malarious area in northwest Argentina. *J of Vector Ecol* 40: 36-45.
- Kahamba NF, Limwagu AJ, Mapua SA, Msugupakulya BJ, Msaky DS, Kaindoa EW, Ngowo HS & Okumu FO (2020) Habitat characteristics and insecticide susceptibility of *Aedes aegypti* in the Ifakara area, south-eastern Tanzania. *Parasit Vectors* 13: 53. doi:10.1186/s13071-020-3920-y.
- Kajeguka DC, Kaaya RD, Mwakalinga S, Ndossi R, Ndaro A, Chilongola JO, Mosha FW, Schiøler KL, Kavishe RA & Alifrangis M (2016) Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *BMC Infectious Diseases* 16: 183. doi:10.1186/s12879-016-1511-5.
- Kamareddine L (2012) The biological control of the malaria vector. *Toxins (Basel)* 4: 748-767. doi:10.3390/toxins4090748.
- Kamau WW, Sang R, Ogola EO, Rotich G, Getugi C, Agha SB, Menza N, Torto B & Tchouassi DP (2022) Survival rate, blood feeding habits and sibling species composition of *Aedes simpsoni* complex: Implications for arbovirus transmission risk in East Africa. *PLoS Negl Trop Dis* 16: e0010171. doi:10.1371/journal.pntd.0010171.
- Kamgang B, Wilson-Bahun TA, Yougang AP, Lenga A & Wondji CS (2020) Contrasting resistance patterns to type I and II pyrethroids in two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in the Republic of the Congo, Central Africa. *Infect Dis Poverty* 9: 23. doi:10.1186/s40249-020-0637-2.
- Kampango A & Abilio AP (2016) The Asian tiger hunts in Maputo city--the first confirmed report of *Aedes (Stegomyia) albopictus* (Skuse, 1895) in Mozambique. *Parasit Vectors* 9: 76. doi:10.1186/s13071-016-1361-4.
- Kampango A, Cuamba N & Charlwood JD (2011) Does moonlight influence the biting behaviour of *Anopheles funestus*? *Med Vet Entomol* 25: 240-246. doi:10.1111/j.1365-2915.2010.00917.x.

- Kampango A, Furu P, Sarath DL, Haji KA, Konradsen F, Schiøler KL, Alifrangis M, Saleh F & Weldon CW (2021a) Risk factors for occurrence and abundance of *Aedes aegypti* and *Aedes bromeliae* at hotel compounds in Zanzibar. *Parasit Vectors* 14: 544. doi:10.1186/s13071-021-05005-9.
- Kampango A, Furu P, Sarath DL, Haji KA, Konradsen F, Schioler KL, Alifrangis M, Weldon C & Saleh F (2021b) Targeted elimination of species-rich larval habitats can rapidly collapse mosquito species arbovirus vector populations in Zanzibar. *Med and Vet Ent*: In press.
- Kampango A, Furu P, Sarath DL, Haji KA, Konradsen F, Schioler KL, Alifrangis M, Weldon C & Saleh F (2021c) Targeted elimination of species-rich larval habitats can rapidly collapse mosquito species arbovirus vector populations in Zanzibar. *Med Vet Entomol* 35: 523-533. doi:10.1111/mve.12525.
- Kampango A, Hocke EF, Hansson H, Furu P, Haji KA, David JP, Konradsen F, Saleh F, Weldon CW, Schioler KL & Alifrangis M (2022) High DDT resistance without apparent association to kdr and Glutathione-S-transferase (GST) gene mutations in *Aedes aegypti* population at hotel compounds in Zanzibar. *PLoS Negl Trop Dis* 16: e0010355. doi:10.1371/journal.pntd.0010355.
- Kapmeier F & Gonçalves P (2018) Wasted paradise? Policies for Small Island States to manage tourism-driven growth while controlling waste generation: the case of the Maldives. *System Dynamics Review* 34: 172-221. doi:<https://doi.org/10.1002/sdr.1607>.
- Kats LB & Ferrer RP (2003) Alien predators and amphibian declines: Review of two decades of science and the transition to conservation. *Diversity distribution* 9: 99-110. doi:10.1046/j.1472-4642.2003.00013.x.
- Kawada H, Higa Y, Futami K, Muranami Y, Kawashima E, Osei JHN, Sakyi KY, Dadzie S, de Souza DK, Appawu M, Ohta N, Suzuki T & Minakawa N (2016) Discovery of Point Mutations in the Voltage-Gated Sodium Channel from African *Aedes aegypti* Populations: Potential Phylogenetic Reasons for Gene Introgression. *PLoS Negl Trop Dis* 10: e0004780. doi:10.1371/journal.pntd.0004780.

- Kawada H, Higa Y, Komagata O, Kasai S, Tomita T, Thi Yen N, Loan LL, Sánchez RAP & Takagi M (2009) Widespread Distribution of a Newly Found Point Mutation in Voltage-Gated Sodium Channel in Pyrethroid-Resistant *Aedes aegypti* Populations in Vietnam. *PLoS Negl Trop Dis* 3: e527. doi:10.1371/journal.pntd.0000527.
- Kawada H, Oo SZM, Thaung S, Kawashima E, Maung YNM, Thu HM, Thant KZ & Minakawa N (2014) Co-occurrence of Point Mutations in the Voltage-Gated Sodium Channel of Pyrethroid-Resistant *Aedes aegypti* Populations in Myanmar. *PLoS Negl Trop Dis* 8: e3032. doi:10.1371/journal.pntd.0003032.
- Keiser J, Singer BH & Utzinger J (2005) Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect Dis* 5: 695-708. doi:10.1016/S1473-3099(05)70268-1.
- Keïta M, Doumbia S, Sissoko I, Touré M, Diawara SI, Konaté D, Sodio AB, Traoré SF, Diakité M, Doumbia SO, Sogoba N, Krogstad DJ, Shaffer JG & Coulibaly MB (2021) Indoor and outdoor malaria transmission in two ecological settings in rural Mali: implications for vector control. *Malar J* 20: 127. doi:10.1186/s12936-021-03650-0.
- Kennedy D & Marjorie L (1999) Rubber, Blight, and Mosquitoes: Biogeography Meets the Global Economy. *Environmental History* 4: 369-383. doi:10.2307/3985132.
- Kesavaraju B & Juliano SA (2004) Differential Behavioral Responses to Water-Borne Cues to Predation in Two Container-Dwelling Mosquitoes. *Ann Entomol Soc Am* 97: 194-201. doi:10.1603/0013-8746(2004)097[0194:dbrtwc]2.0.co;2.
- Khallaayoune K, Qualls WA, Revay EE, Allan SA, Arheart KL, Kravchenko VD, Xue R-D, Schlein Y, Beier JC & Müller GC (2013) Attractive Toxic Sugar Baits: Control of Mosquitoes With the Low-Risk Active Ingredient Dinotefuran and Potential Impacts on Nontarget Organisms in Morocco. *Environ Entomol* 42: 1040-1045. doi:10.1603/EN13119.
- Killeen GF (2014) Characterizing, controlling and eliminating residual malaria transmission. *Malar J* 13: 330. doi:10.1186/1475-2875-13-330.

- Killeen GF, Fillinger U, Kiche I, Gouagna LC & Knols BGJ (2002) Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *The Lancet Infectious Diseases* 2: 618-627. doi:10.1016/S1473-3099(02)00397-3.
- Killeen GF, Smith TA, Ferguson HM, Mshinda H, Abdulla S, Lengeler C & Kachur SP (2007) Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. *PLoS Med* 4: e229. doi:10.1371/journal.pmed.0040229.
- Kinimi E, Shayo MJ, Patrick BN, Angwenyi SA-O, Kasanga CJ, Weyer J, Jansen van Vuren PA-O, Paweska JA-O, Mboera LEG & Misinzo GA-O (2018) Evidence of chikungunya virus infection among febrile patients seeking healthcare in selected districts of Tanzania. *Infect Ecol Epidemiol* 8: 1553460.
- Klowden MJ (1995) Blood, Sex and the Mosquito. *BioSciences* 45: 326-331.
- Knipling EF (1959) Sterile-Male Method of Population Control. *Science* 130: 902-904. doi:doi:10.1126/science.130.3380.902.
- Knox TB, Yen NT, Nam VS, Gatton ML, Kay BH & Ryan PA (2007) Critical evaluation of quantitative sampling methods for *Aedes aegypti* (Diptera: Culicidae) immatures in water storage containers in Vietnam. *J Med Entomol* 44: 192-204. doi:10.1603/0022-2585(2007)44[192:ceoqsm]2.0.co;2.
- Koenraadt CJM & Harrington LC (2008) Flushing Effect of Rain on Container-Inhabiting Mosquitoes *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae). *J Med Entomol* 45: 28-35. doi:10.1093/jmedent/45.1.28.
- Konradsen F, van der Hoek W, Amerasinghe FP, Mutero C & Boelee E (2004) Engineering and malaria control: learning from the past 100 years. *Acta Trop* 89: 99-108. doi:10.1016/j.actatropica.2003.09.013.
- Kraemer MUG, Reiner RC, Jr., Brady OJ, Messina JP, Gilbert M, Pigott DM, Yi D, Johnson K, Earl L, Marczak LB, Shirude S, Davis Weaver N, Bisanzio D, Perkins TA, Lai S, Lu X, Jones P, Coelho GE, Carvalho RG, Van Bortel W, Marsboom C, Hendrickx G, Schaffner F, Moore CG, Nax HH, Bengtsson L, Wetter E, Tatem AJ, Brownstein JS, Smith DL, Lambrechts L, Cauchemez S, Linard C, Faria NR, Pybus OG, Scott TW, Liu Q, Yu H, Wint GRW, Hay SI & Golding N (2019) Past and future spread of the

- arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat Microbiol* 4: 854-863. doi:10.1038/s41564-019-0376-y.
- Kukkonen MO, Muhammad MJ, Käyhkö N & Luoto M (2018) Urban expansion in Zanzibar City, Tanzania: Analyzing quantity, spatial patterns and effects of alternative planning approaches. *Land Use Policy* 71: 554-565.
- Kumar G, Pande V, Pasi S, Ojha VP & Dhiman RC (2018a) Air versus water temperature of aquatic habitats in Delhi: Implications for transmission dynamics of *Aedes aegypti*. *Geospat Health* 13. doi:10.4081/gh.2018.707.
- Kumar S, Stecher G, Li M, Knyaz C & Tamura K (2018b) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular biology and evolution* 35: 1547-1549. doi:10.1093/molbev/msy096.
- Kweka EJ, Kimaro EE & Munga S (2016) Effect of Deforestation and Land Use Changes on Mosquito Productivity and Development in Western Kenya Highlands: Implication for Malaria Risk. *Frontiers in public health* 4: 238-238. doi:10.3389/fpubh.2016.00238.
- Kyalo D, Amratia P, Mundia CW, Mbogo CM, Coetzee M & Snow RW (2017) A geo-coded inventory of anophelines in the Afrotropical Region south of the Sahara: 1898-2016. *Wellcome Open Res* 2: 57. doi:10.12688/wellcomeopenres.12187.1.
- LaDeau SL, Allan BF, Leisnham PT & Levy MZ (2015) The ecological foundations of transmission potential and vector-borne disease in urban landscapes. *Functional ecology* 29: 889-901. doi:10.1111/1365-2435.12487.
- Laird M (1988) *The natural history of laval mosquito habitats*. Academic Press, Ltd, London, UK.
- Lami F, Bartomeus I, Nardi D, Beduschi T, Boscutti F, Pantini P, Santoiemma G, Scherber C, Tschardt T & Marini L (2021) Species-habitat networks elucidate landscape effects on habitat specialisation of natural enemies and pollinators. *Ecol Lett* 24: 288-297. doi:10.1111/ele.13642.

- Le Menach A, Tatem AJ, Cohen JM, Hay SI, Randell H, Patil AP & Smith DL (2011) Travel risk, malaria importation and malaria transmission in Zanzibar. *Sci Rep* 1: 93. doi:10.1038/srep00093.
- Leal WS (2014) The enigmatic reception of DEET—the gold standard of insect repellents. *Curr Opin Insect Sci* 6: 93-98. doi:<https://doi.org/10.1016/j.cois.2014.10.007>.
- Lehane MJ (2005) *Biology of Blood-sucking Insects*. Cambridge University Press, Cambridge, United Kingdom.
- Li C-X, Kaufman PE, Xue RD, Zhao MH, Wang G, Yan T, Guo XX, Zhang YM, Dong YD, Xing D, Zhang HD & Zhao TY (2015) Relationship between insecticide resistance and kdr mutations in the dengue vector *Aedes aegypti* in Southern China. *Parasit Vectors* 8.
- Liew C & Curtis CF (2004) Horizontal and vertical dispersal of dengue vector mosquitoes, *Aedes aegypti* and *Aedes albopictus*, in Singapore. *Med Vet Entomol* 18: 351-360. doi:<https://doi.org/10.1111/j.0269-283X.2004.00517.x>.
- Lindsay S, Kirby MJ, Baris E & Bos R (2004) Environmental management for malaria control in the East Asia and Pacific (EAP) region: World Bank Health, Nutrition and Population Discussion Paper (ed. by AS Preker) The World Bank.
- Lines JD & Nassor NS (1991) DDT resistance in *Anopheles gambiae* declines with mosquito age. *Med and Vet Ent* 5: 261-265.
- Linss JGB, Brito LP, Garcia GA, Araki AS, Bruno RV, Lima JBP, Valle D & Martins AJ (2014) Distribution and dissemination of the Val1016Ile and Phe1534Cys Kdr mutations in *Aedes aegypti* Brazilian natural populations. *Parasit Vectors* 7: 25. doi:10.1186/1756-3305-7-25.
- Linthicum KJ, Britch SC & Anyamba A (2016) Rift Valley Fever: An Emerging Mosquito-Borne Disease. *Annu Rev Entomol* 61: 395-415. doi:10.1146/annurev-ento-010715-023819.
- Liu-Helmersson J, Quam M, Wilder-Smith A, Stenlund H, Ebi K, Massad E & Rocklöv J (2016) Climate Change and *Aedes* Vectors: 21st Century Projections for Dengue

Transmission in Europe. *EBioMedicine* 7: 267-277.  
doi:<https://doi.org/10.1016/j.ebiom.2016.03.046>.

Liu N (2015) Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu Rev Entomol* 60: 537-559. doi:10.1146/annurev-ento-010814-020828.

Loding Magazine (2019) Hyatt to Reduce Plastic Waste With Bulk Bathroom Amenities and Water Stations, Vol. 2022.

Lounibos LP (1980) The bionomics of three sympatric Eretmapodites (Diptera: Culicidae) at the Kenya coast. *Bull Ent Res* 70: 309-320.

Lounibos PL (2002) Invasions by insect vectors of human diseases. *Annual Rev Ent* 47: 233-263.

Lowe R, Gasparrini A, Van Meerbeeck CJ, Lippi CA, Mahon R, Trotman AR, Rollock L, Hinds AQJ, Ryan SJ & Stewart-Ibarra AM (2018) Nonlinear and delayed impacts of climate on dengue risk in Barbados: A modelling study. *PLoS Med* 15: e1002613. doi:10.1371/journal.pmed.1002613.

Lucas JR, Shono Y, Iwasaki T, Ishiwatari T, Spero N & Benzon G (2007) U.S. laboratory and field trials of metofluthrin (SumiOne) emanators for reducing mosquito biting outdoors. *J Am Mosq Control Assoc* 23: 47-54. doi:10.2987/8756-971x(2007)23[47:ulafto]2.0.co;2.

Lucey D & Gostin LO (2016) A Yellow Fever Epidemic: A New Global Health Emergency? *JAMA* 315: 2661-2662. doi:10.1001/jama.2016.6606.

Lüdecke D, Makowski D, Waggoner P & Patil I (2020) Assessment of Regression Models Performance. R package version 0.6.1. <https://easystats.github.io/performance>.

MacDonald AJ & Mordecai EA (2019) Amazon deforestation drives malaria transmission, and malaria burden reduces forest clearing. *Proceedings of the National Academy of Sciences* 116: 22212. doi:10.1073/pnas.1905315116.

- Madon MB, Mulla MS, Shaw MW, Kluh S & Hazelrigg JE (2002) Introduction of *Aedes albopictus* (Skuse) in southern California and potential for its establishment. *J Vector Ecol* 27: 149-154.
- Main BJ, Lee Y, Ferguson HM, Kreppel KS, Kihonda A, Govella NJ, Collier TC, Cornel AJ, Eskin E, Kang EY, Nieman CC, Weakley AM & Lanzaro GC (2016) The Genetic Basis of Host Preference and Resting Behavior in the Major African Malaria Vector, *Anopheles arabiensis*. *PLoS Genet* 12: e1006303. doi:10.1371/journal.pgen.1006303.
- Manguin S, Bangs MJ, Pothikasikorn J & Chareonviriyaphap T (2010) Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. *Infect Genet Evol* 10: 159-177. doi:10.1016/j.meegid.2009.11.014.
- Manrique-Saide P, Che-Mendoza A, Rizzo N, Arana B, Pilger D, Lenhart A & Kroeger A (2011) Operational guide for assessing the productivity of *Aedes aegypti* breeding sites. World Health Organization, Geneva, Switzerland.
- Marcantonio M, Metz M, Baldacchino F, Arnoldi D, Montarsi F, Capelli G, Carlin S, Neteler M & Rizzoli A (2016) First assessment of potential distribution and dispersal capacity of the emerging invasive mosquito *Aedes koreicus* in Northeast Italy. *Parasit Vectors* 9: 63. doi:10.1186/s13071-016-1340-9.
- Marcantonio M, Reyes T & Barker CM (2019) Quantifying *Aedes aegypti* dispersal in space and time: a modeling approach. *Ecosphere* 10: 1-18.
- Marini L, Bartomeus I, Rader R & Lami F (2018) Species–habitat networks: A tool to improve landscape management for conservation. *J Appl Ecol* 56: 923–928.
- Martel V, Schlyter F, Ignell R, Hansson BS & Anderson P (2011) Mosquito Feeding Affects Larval Behaviour and Development in a Moth. *Plos one* 6: e25658. doi:10.1371/journal.pone.0025658.
- Martins AJ, Lima JBP, Peixoto AA & Valle D (2009) Frequency of Val1016Ile mutation in the voltage-gated sodium channel gene of *Aedes aegypti* Brazilian populations. *Tropical Medicine & International Health* 14: 1351-1355. doi:<https://doi.org/10.1111/j.1365-3156.2009.02378.x>.

- Mary BC, Rebekah CK, John-Paul M, Julius JL & Barry RM (2013) Identification of host blood from engorged mosquito collected in western Uganda using cytochrome oxidase I gene sequences. *Journal of Wildlife Diseases* 49: 611-626. doi:10.7589/2012-08-213.
- Mathews G (2011) *Integrated Vector Management: Controlling Vectors of Malaria and Other Insect Borne Disease*. Wiley-Blackwell.
- Mathias L, Baraka V, Philbert A, Innocent E, Francis F, Nkwengulila G & Kweka EJ (2017) Habitat productivity and pyrethroid susceptibility status of *Aedes aegypti* mosquitoes in Dar es Salaam, Tanzania. *Infectious Diseases of Poverty* 6: 102. doi:10.1186/s40249-017-0316-0.
- Maxwell CA, Curtis CF, Haji H, Kisumku S, Thalib AI & Yahya SA (1990) Control of Bancroftian filariasis by integrating therapy with vector control using polystyrene beads in wet pit latrines. *Trans R Soc Trop Med Hyg* 84: 709-714. doi:10.1016/0035-9203(90)90158-b.
- Mayer SV, Tesh RB & Vasilakis N (2017) The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. *Acta Tropica* 166: 155-163. doi:<https://doi.org/10.1016/j.actatropica.2016.11.020>.
- Mboera LE, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, Misinzo G, De Nardo P, Vairo F & Oriyo NM (2016) The Risk of Dengue Virus Transmission in Dar es Salaam, Tanzania during an Epidemic Period of 2014. *PLoS Negl Trop Dis* 10: e0004313. doi:10.1371/journal.pntd.0004313.
- McClelland GAH & Weitz B (1960) Further Observations on the Natural Hosts of Three Species of *Mansonia* Blanchard (Diptera, Culicidae) in Uganda. *Annals of Tropical Medicine & Parasitology* 54: 300-304. doi:10.1080/00034983.1960.11685990.
- McCrae AWR, Boreham PFL & Ssenkubuge Y (1976) The behavioural ecology of host selection in *Anopheles implexus* (Theobald) (Diptera, Culicidae). *Bull Entomol Res* 66: 587 - 631.
- Medeiros-Sousa AR, de Oliveira-Christe R, Camargo AA, Scinachi CA, Milani GM, Urbinatti PR, Natal D, Ceretti-Junior W & Marrelli MT (2020) Influence of water's physical and chemical parameters on mosquito (Diptera: Culicidae) assemblages in larval habitats in

urban parks of São Paulo, Brazil. *Acta Tropica* 205: 105394.  
doi:<https://doi.org/10.1016/j.actatropica.2020.105394>.

Memmott J, Waser NM & Price MV (2004) Tolerance of pollination networks to species extinctions. *Proc Roy Soc B* 271: 2605 - 2611.

Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, Pigott DM, Shearer FM, Johnson K, Earl L, Marczak LB, Shirude S, Davis Weaver N, Gilbert M, Velayudhan R, Jones P, Jaenisch T, Scott TW, Reiner RC & Hay SI (2019) The current and future global distribution and population at risk of dengue. *Nat Microbiol* 4: 1508-1515. doi:10.1038/s41564-019-0476-8.

Meyers JJ, Pathikonda S, Popkin-Hall ZR, Medeiros MC, Fuseini G, Matias A, Garcia G, Overgaard HJ, Kulkarni V, Reddy VP, Schwabe C, Lines J, Kleinschmidt I & Slotman MA (2016) Increasing outdoor host-seeking in *Anopheles gambiae* over 6 years of vector control on Bioko Island. *Malar J* 15: 239. doi:10.1186/s12936-016-1286-6.

Milali MP, Sikulu-Lord MT & Govella NJ (2017) Bites before and after bedtime can carry a high risk of human malaria infection. *Malar J* 16: 91. doi:10.1186/s12936-017-1740-0.

Mmbando AS, Ngowo H, Limwagu A, Kilalangongono M, Kifungo K & Okumu FO (2018) Eave ribbons treated with the spatial repellent, transfluthrin, can effectively protect against indoor-biting and outdoor-biting malaria mosquitoes. *Malar J* 17: 368. doi:10.1186/s12936-018-2520-1.

Moiroux N, Damien GB, Egrot M, Djenontin A, Chandre F, Corbel V, Killeen GF & Pennetier C (2014) Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets. *Plos one* 9: e104967. doi:10.1371/journal.pone.0104967.

Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, Djegbe I, Guis H & Corbel V (2012) Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *J Infect Dis* 206: 1622-1629. doi:10.1093/infdis/jis565.

Mordecai EA, Caldwell JM, Grossman MK, Lippi CA, Johnson LR, Neira M, Rohr JR, Ryan SJ, Savage V, Shocket MS, Sippy R, Stewart Ibarra AM, Thomas MB & Villena O

- (2019) Thermal biology of mosquito-borne disease. *Ecology Letters* 22: 1690-1708. doi:<https://doi.org/10.1111/ele.13335>.
- Mordecai EA, Paaijmans KP, Johnson LR, Balzer C, Ben-Horin T, Moor E, McNally A, Pawar S, Ryan SJ, Smith TC & Lafferty KD (2013) Optimal temperature for malaria transmission is dramatically lower than previously predicted. *Ecology Letters* 16: 22-30.
- Mordecai EA, Ryan SJ, Caldwell JM, Shah MM & LaBeaud AD (2020) Climate change could shift disease burden from malaria to arboviruses in Africa. *Lancet Planet Health* 4: 416-423.
- Morgan AP, Brazeau NF, Ngasala B, Mhamilawa LE, Denton M, Msellem M, Morris U, Filer DL, Aydemir O, Bailey JA, Parr JB, Martensson A, Bjorkman A & Juliano JJ (2020) *Falciparum* malaria from coastal Tanzania and Zanzibar remains highly connected despite effective control efforts on the archipelago. *Malar J* 19: 47. doi:10.1186/s12936-020-3137-8.
- Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R & Schäffer AA (2008) Database indexing for production MegaBLAST searches. *Bioinformatics* 24: 1757-1764. doi:10.1093/bioinformatics/btn322.
- Morrison AC, Reiner RC, Jr., Elson WH, Astete H, Guevara C, Del Aguila C, Bazan I, Siles C, Barrera P, Kawiecki AB, Barker CM, Vasquez GM, Escobedo-Vargas K, Flores-Mendoza C, Huaman AA, Leguia M, Silva ME, Jenkins SA, Campbell WR, Abente EJ, Hontz RD, Paz-Soldan VA, Grieco JP, Lobo NF, Scott TW & Achee NL (2022) Efficacy of a spatial repellent for control of *Aedes*-borne virus transmission: A cluster-randomized trial in Iquitos, Peru. *Proc Natl Acad Sci U S A* 119: e2118283119. doi:10.1073/pnas.2118283119.
- Mota-Sanchez D & Wise JC (2019) *The Arthropod Pesticide Resistance Database.*, Vol. 2019: Michigan State University.
- Mouchet J, Carnevale P, Coosemans M, Julvez J, Manguin S, Richard-Lenoble D & Sircoulon J (2004) *Biodiversité du paludisme dans le monde*. John Libbey Eurotext, Paris, France.

- Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David J-P & Weetman D (2017) Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Negl Trop Dis* 11: e0005625. doi:10.1371/journal.pntd.0005625.
- Muhammad NAF, Abu Kassim NF, Ab Majid AH, Abd Rahman A, Dieng H & Avicor SW (2020) Biting rhythm and demographic attributes of *Aedes albopictus* (Skuse) females from different urbanized settings in Penang Island, Malaysia under uncontrolled laboratory conditions. *Plos one* 15: e0241688. doi:10.1371/journal.pone.0241688.
- Muirhead-Thomson RC (2009) Studies on Salt-Water and Fresh-Water *Anopheles gambiae* on the East African Coast. *Bull Entomol Res* 41: 487-502. doi:10.1017/S0007485300027772.
- Mukhtar MU, Han Q, Liao C, Haq F, Arslan A & Bhatti A (2018) Seasonal Distribution and Container Preference Ratio of the Dengue Fever Vector (*Aedes aegypti*, Diptera: Culicidae) in Rawalpindi, Pakistan. *J Med Entomol* 55: 1011-1015. doi:10.1093/jme/tjy010.
- Muller GC, Junnila A, Qualls W, Revay EE, Kline DL, Allan S, Schlein Y & Xue RD (2010) Control of *Culex quinquefasciatus* in a storm drain system in Florida using attractive toxic sugar baits. *Med Vet Entomol* 24: 346-351. doi:10.1111/j.1365-2915.2010.00876.x.
- Murcia O, Henríquez B, Castro A, Koo S, Young J, Márquez R, Pérez D, Cáceres L & Valderrama A (2019) Presence of the point mutations Val1016Gly in the voltage-gated sodium channel detected in a single mosquito from Panama. *Parasit Vectors* 12: 62. doi:10.1186/s13071-019-3309-y.
- Murdock CC, Sternberg ED & Thomas MB (2016) Malaria transmission potential could be reduced with current and future climate change. *Sci Rep* 6: 27771. doi:10.1038/srep27771.
- Mwalusepo S, Muli E, Faki A & Raina S (2017) Land use and land cover data changes in Indian Ocean Islands: Case study of Unguja in Zanzibar Island. *Data Brief* 11: 117-121. doi:10.1016/j.dib.2017.01.010.

- Mwanyika GO, Mboera LEG, Rugarabamu S, Makange M, Sindato C, Lutwama JJ, Paweska JT & Misinzo G (2021) Circulation of dengue serotype 1 viruses during the 2019 outbreak in Dar es Salaam, Tanzania. *Pathog Glob Health* 115: 467-475. doi:10.1080/20477724.2021.1905302.
- Mweya CN, Kimera SI, Stanley G, Misinzo G & Mboera LE (2016) Climate Change Influences Potential Distribution of Infected *Aedes aegypti* Co-Occurrence with Dengue Epidemics Risk Areas in Tanzania. *Plos one* 11: e0162649. doi:10.1371/journal.pone.0162649.
- Nanfack Minkeu F & Vernick KD (2018) A Systematic Review of the Natural Virome of *Anopheles* Mosquitoes. *Viruses* 10. doi:10.3390/v10050222.
- Nataša B, Mladen K, Enrih M & Branka B-M (2021) Diversity of mosquito fauna (Diptera, Culicidae) in higher-altitude regions of Croatia. *Journal of Vector Ecology* 46: 65-75. doi:10.52707/1081-1710-46.1.65.
- Ndiath MO (2019) Insecticides and Insecticide Resistance. *Methods in Molecular Biology* 2013: 287-304.
- Ngoagouni C, Kamgang B, Nakouné E, Paupy C & Kazanji M (2015) Invasion of *Aedes albopictus* (Diptera: Culicidae) into central Africa: what consequences for emerging diseases? *Parasit Vectors* 8: 191. doi:10.1186/s13071-015-0808-3.
- Nkahe DL, Kopya E, Djiappi-Tchamen B, Toussile W, Sonhafouo-Chiana N, Kekeunou S, Mimpfoundi R, Awono-Ambene P, Wondji CS & Antonio-Nkondjio C (2020) Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaounde, Cameroon. *Wellcome Open Res* 5: 171. doi:10.12688/wellcomeopenres.16039.2.
- Norris DE (2004) Mosquito-borne disease as a consequence of land use. *EcoHealth* 1: 19-24.
- Nyamwaya DK, Otiende M, Omuoyo DO, Githinji G, Karanja HK, Gitonga JN, R. de Laurent Z, Otieno JR, Sang R, Kamau E, Cheruiyot S, Otieno E, Agoti CN, Bejon P, Thumbi SM & Warimwe GM (2021) Endemic chikungunya fever in Kenyan children: a prospective cohort study. *BMC Infectious Diseases* 21: 186. doi:10.1186/s12879-021-05875-5.

- Office of Chief Government Statistician-Zanzibar (2020) Hotels and Tourism Statistics: Office of Chief Government Statistician-Zanzibar.
- Ogoma SB, Lweitoijera DW, Ngonyani H, Furer B & Russell TL (2010) Screening mosquito house entry points as a potential method for integrated control of endophagic filariasis, arbovirus and malaria vectors. *PLoS Negl Trop Dis* 4: e773.
- Ogoma SB, Mmando AS, Swai JK, Horstmann S, Malone D & Killeen GF (2017) A low technology emanator treated with the volatile pyrethroid transfluthrin confers long term protection against outdoor biting vectors of lymphatic filariasis, arboviruses and malaria. *PLoS Negl Trop Dis* 11: e0005455. doi:10.1371/journal.pntd.0005455.
- Osorio HC, Ze-Ze L & Alves MJ (2012) Host-feeding patterns of *Culex pipiens* and other potential mosquito vectors (Diptera: Culicidae) of West Nile virus (Flaviviridae) collected in Portugal. *J Med Entomol* 49: 717-721. doi:10.1603/me11184.
- Ovaskaine O & Abrego N (2020) Joint Species Distribution Modelling - with Applications in R Cambridge University Press.
- Ovaskainen O, Tikhonov G, Norberg A, Guillaume Blanchet F, Duan L, Dunson D, Roslin T & Abrego N (2017) How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecology Letters* 20: 561-576. doi:<https://doi.org/10.1111/ele.12757>.
- Paaijmans KP, Blanford S, Chan BHK & Thomas MB (2012) Warmer temperatures reduce the vectorial capacity of malaria mosquitoes. *Biol Lett* 8: 465-468. doi:10.1098/rsbl.2011.1075.
- Paaijmans KP, Wandago MO, Githeko AK & Takken W (2007) Unexpected high losses of *Anopheles gambiae* larvae due to rainfall. *Plos one* 2: e1146. doi:10.1371/journal.pone.0001146.
- Paixão ES, Teixeira MG & Rodrigues LC (2018) Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. *BMJ Glob Health* 3: e000530-e000530. doi:10.1136/bmjgh-2017-000530.

- Pang T, Mak TK & Gubler DJ (2017) Prevention and control of dengue-the light at the end of the tunnel. *The Lancet Infectious Diseases* 17: e79-e87. doi:10.1016/S1473-3099(16)30471-6.
- Parola P, de Lamballerie X, Jourdan J, Rovey C, Vaillant V, Minodier P, Brouqui P, Flahault A, Raoult D & Charrel RN (2006) Novel chikungunya virus variant in travelers returning from Indian Ocean islands. *Emerg Infect Dis* 12: 1493-1499. doi:10.3201/eid1210.060610.
- Patefield WM (1981) Algorithm AS 159: An Efficient Method of Generating Random  $R \times C$  Tables with Given Row and Column Totals. *Appl Stats* 30: 91-97.
- Peach DAH & Gries G (2020) Mosquito phytophagy – sources exploited, ecological function, and evolutionary transition to haematophagy. *Entomologia Experimentalis et Applicata* 168: 120-136. doi:<https://doi.org/10.1111/eea.12852>.
- Pettorelli N, Vik JO, Myrsterud A, Gaillard JM, Tucker CJ & Stenseth NC (2005) Using the satellite-derived NDVI to assess ecological responses to environmental change. *Trends Ecol Evol* 20: 503-510. doi:10.1016/j.tree.2005.05.011.
- Poulin B (2011) Indirect effects of bioinsecticides on the nontarget fauna: The Camargue experiment calls for future research. *Acta Oecologica* 44: 28-32.
- Powell JR (2016) Mosquitoes on the move. *Science* 354: 971. doi:10.1126/science.aal1717.
- Prapanthadara L-a, Hemingway J & Ketterman AJ (2009) DDT-resistance in *Anopheles gambiae* (Diptera: Culicidae) from Zanzibar, Tanzania, based on increased DDT-dehydrochlorinase activity of glutathione S-transferases. *Bull Entomol Res* 85: 267-274. doi:10.1017/S0007485300034350.
- Prapanthadara L, Hemingway J & Ketterman A (1995) DDT-resistance in *Anopheles gambiae* (Diptera: Culicidae) from Zanzibar, Tanzania, based on increased DDT-dehydrochlorinase activity of glutathione S-transferases. *Bull Ent Res* 85: 267-274. doi:10.1017/S0007485300034350.
- Provost MW (1952) Motives behind mosquito flights. *Mosquito News* 13: 106-109.

- Puntasecca CJ, King CH & LaBeaud AD (2021) Measuring the global burden of chikungunya and Zika viruses: A systematic review. *PLoS Negl Trop Dis* 15: e0009055. doi:10.1371/journal.pntd.0009055.
- R Core Team (2022) R: A language and environment for statistical computing: R Foundation for Statistical Computing, Vienna, Austria.
- Raghavendra K, Verma V, Srivastava HC, Gunasekaran K, Sreehari U & Dash AP (2010) Persistence of DDT, malathion & deltamethrin resistance in *Anopheles culicifacies* after their sequential withdrawal from indoor residual spraying in Surat district, India. *The Indian journal of medical research* 132: 260-264.
- Rebollo MP, Mohammed KA, Thomas B, Ame S, Ali SM, Cano J, Escalada AG & Bockarie MJ (2015) Cessation of mass drug administration for lymphatic filariasis in Zanzibar in 2006: was transmission interrupted? *PLoS Negl Trop Dis* 9: e0003669. doi:10.1371/journal.pntd.0003669.
- Rehwagen C (2006) WHO recommends DDT to control malaria. *BMJ (Clinical research ed.)* 333: 622-622. doi:10.1136/bmj.333.7569.622-b.
- Reinert JF & Harbach RE (2005) Generic and subgeneric status of aedine mosquito species (Diptera: Culicidae: Aedini) occurring in the Australasian Region. *Zootaxa* 887: 1-10.
- Reiter P (2007) Oviposition, dispersal, and survival in *Aedes aegypti*: implications for the efficacy of control strategies. *Vector Borne Zoonotic Dis* 7: 261-273. doi:10.1089/vbz.2006.0630.
- Reiter P (2008) Climate change and mosquito-borne disease: knowing the horse before hitching the cart. *Rev Sci Tech* 27: 383-398.
- Reiter P & Darsie RF, Jr. (1984) *Aedes albopictus* in Memphis, Tennessee (USA): an achievement of modern transportation. *Mosquito News* 44: 396-399.
- Rejmánková E, Grieco J, Achee NR & Roberts D (2013) Ecology of Larval Habitats: *Anopheles* mosquitoes - New insights into malaria vectors (ed. by S Manguin) IntechOpen.

- Rejmánková E, Higashi R, Grieco J, Achee N & Roberts D (2005) Volatile Substances from Larval Habitats Mediate Species-Specific Oviposition in Anopheles Mosquitoes. *J Med Entomol* 42: 95-103. doi:10.1093/jmedent/42.2.95.
- Rezende GL, Martins AJ, Gentile C, Farnesi LC, Pelajo-Machado M, Peixoto AA & Valle D (2008) Embryonic desiccation resistance in *Aedes aegypti*: presumptive role of the chitinized serosal cuticle. *BMC developmental biology* 8: 82-82. doi:10.1186/1471-213X-8-82.
- Riffell JA (2019) Olfaction: Repellents that Congest the Mosquito Nose. *Current Biology* 29: R1124-R1126. doi:10.1016/j.cub.2019.09.053.
- Ritz C & Streibig JC (2005) Bioassay Analysis using R. *J Statist Software* 12.
- Rocklov J & Dubrow R (2020) Climate change: an enduring challenge for vector-borne disease prevention and control. *Nature Immunology* 21: 479 - 483.
- Rodríguez-Rodríguez D, Katusele M, Auwun A, Marem M, Robinson LJ, Laman M, Hetzel MW & Pulford J (2021) Human Behavior, Livelihood, and Malaria Transmission in Two Sites of Papua New Guinea. *J Infect Dis* 223: S171-S186. doi:10.1093/infdis/jiaa402.
- Roiz D, Ruiz S, Soriguer R & Figuerola J (2015) Landscape Effects on the Presence, Abundance and Diversity of Mosquitoes in Mediterranean Wetlands. *Plos one* 10: e0128112. doi:10.1371/journal.pone.0128112.
- Rose NH, Sylla M, Badolo A, Lutomiah J, Ayala D, Aribodor OB, Ibe N, Akorli J, Otoo S, Mutebi J-P, Kriete AL, Ewing EG, Sang R, Gloria-Soria A, Powell JR, Baker RE, White BJ, Crawford JE & McBride CS (2020) Climate and Urbanization Drive Mosquito Preference for Humans. *Current Biology* 30: 3570-3579.e3576. doi:<https://doi.org/10.1016/j.cub.2020.06.092>.
- Rossello J, Santana-Gallego M & Awan W (2017) Infectious disease risk and international tourism demand. *Health Policy Plan* 32: 538-548. doi:10.1093/heapol/czw177.
- Rozendaal JA (1997) Vector Control: Methods for use by individuals and communities. World Health Organization, Geneva, Switzerland.

- Rueda LM (2008) Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. *Hydrobiologia* 595: 477-487.
- Russo G, Subissi L & Rezza G (2020) Chikungunya fever in Africa: a systematic review. *Pathogens and Global Health* 114: 111-119. doi:10.1080/20477724.2020.1748965.
- Ryan SJ, Carlson CJ, Mordecai EA & Johnson LR (2019) Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. *PLoS Negl Trop Dis* 13: e0007213. doi:10.1371/journal.pntd.0007213.
- Ryan SJ, Lippi CA & Zermoglio F (2020) Shifting transmission risk for malaria in Africa with climate change: a framework for planning and intervention. *Malar J* 19: 170. doi:10.1186/s12936-020-03224-6.
- Saleh F, Kitau J, Konradsen F, Alifrangis M, Lin C-H, Juma S, Mchenga SS, Saadaty T & Schioler KL (2018) Habitat characteristic for immature Stages of Aedes aegypti in Zanzibar City, Tanzania. *J Am Mosq Contr Ass* 34: 190-200.
- Saleh F, Kitau J, Konradsen F, Kampango A, Abassi R & Schiøler KL (2020a) Epidemic risk of arboviral diseases: Determining the habitats, spatial-temporal distribution, and abundance of immature Aedes aegypti in the Urban and Rural areas of Zanzibar, Tanzania. *PLoS Negl Trop Dis* 14: e0008949. .
- Saleh F, Kitau J, Konradsen F, Kampango A, Abassi R & Schiøler KL (2020b) Epidemic risk of arboviral diseases: Determining the habitats, spatial-temporal distribution, and abundance of immature Aedes aegypti in the Urban and Rural areas of Zanzibar, Tanzania. *PLoS Negl Trop Dis* 14: e0008949.
- Saleh F, Kitau J, Konradsen F, Mboera LEG & Schioler KL (2021) Emerging epidemics: is the Zanzibar healthcare system ready to detect and respond to mosquito-borne viral diseases? *BMC Health Serv Res* 21: 866. doi:10.1186/s12913-021-06867-6.
- Saleh MS, El-Meniawi FA, Kelada NL & Zahran HM (2003) Resistance development in mosquito larvae Culex pipiens to the bacterial agent Bacillus thuringiensis var. israelensis. *Journal of Applied Entomology* 127: 29-32. doi:<https://doi.org/10.1046/j.1439-0418.2003.00703.x>.

- Sánchez-Bayo F & Wyckhuys KAG (2019) Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation* 232: 8-27. doi:<https://doi.org/10.1016/j.biocon.2019.01.020>.
- Sangbakembi-Ngounou C, Costantini C, Longo-Pendy NM, Ngoagouni C, Akone-Ella O, Rahola N, Cornelie S, Kengne P, Nakouné ER, Komas NP & Ayala D (2022) Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be out of control. *Proceedings of the National Academy of Sciences* 119: e2104282119. doi:[doi:10.1073/pnas.2104282119](https://doi.org/10.1073/pnas.2104282119).
- Sauer FG, Grave J, Lühken R & Kiel E (2021) Habitat and microclimate affect the resting site selection of mosquitoes. *Med Vet Entomol* n/a. doi:<https://doi.org/10.1111/mve.12506>.
- Schneider P, Takken W & McCall PJ (2000) Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *An. gambiae*. *Med Vet Entomol* 14: 165-170.
- Schwartz E, Pener H, Issa SM & Golenser J (1997) An Overview of the Malaria Situation in Zanzibar. *Journal of Community Health* 22: 33-44. doi:[10.1023/A:1025194823592](https://doi.org/10.1023/A:1025194823592).
- Sene NM, Mavridis K, Ndiaye EH, Diagne CT, Gaye A, Ngom EHM, Ba Y, Diallo D, Vontas J, Dia I & Diallo M (2021) Insecticide resistance status and mechanisms in *Aedes aegypti* populations from Senegal. *PLoS Negl Trop Dis* 15: e0009393. doi:[10.1371/journal.pntd.0009393](https://doi.org/10.1371/journal.pntd.0009393).
- Service MW (1980) Effect of wind on the behaviour and distribution of mosquitoes and blackflies. *Int J Biometeorol* 24: 347-353.
- Service MW (1990) Handbook to the Afrotropical Toxorhynchitine and Culicine mosquitoes, excepting *Aedes* and *Culex*. British Museum (Natural History), London, UK.
- Service MW (1993) Mosquitoes (Culicidae): Medical Insects and Arachnids (ed. by RP Lane & RW Crosskey) Chapman & Hall.
- Service MW (1997) Mosquito (Diptera: Culicidae) Dispersal—The Long and Short of it. *J Med Entomol* 34: 579-588. doi:[10.1093/jmedent/34.6.579](https://doi.org/10.1093/jmedent/34.6.579).
- Shaman J & Day JF (2007) Reproductive phase locking of mosquito populations in response to rainfall frequency. *Plos one* 2: e331. doi:[10.1371/journal.pone.0000331](https://doi.org/10.1371/journal.pone.0000331).

- Shauri HS, Ngadaya E, Senkoro M, Buza JJ & Mfinanga S (2021) Seroprevalence of Dengue and Chikungunya antibodies among blood donors in Dar es Salaam and Zanzibar, Tanzania: a cross-sectional study. *BMC Infectious Diseases* 21: 911. doi:10.1186/s12879-021-06549-y.
- Shi MA, Lougarre A, Alies C, Fremaux I, Tang ZH, Stojan J & Fournier D (2004) Acetylcholinesterase alterations reveal the fitness cost of mutations conferring insecticide resistance. *BMC Evol Biol* 4: 5. doi:10.1186/1471-2148-4-5.
- Shocket MS, Anderson CB, Caldwell JM, Childs ML, Couper LI, Han S, Harris MJ, Howard ME, Kain MP, MacDonald AJ, Nova N & Mordecai EA (2021) Environmental Drivers of Vector-Borne Diseases: Population Biology of Vector-Borne Diseases. (ed. by JM Drake, MB Bonsall & MR Strand) Oxford University Press.
- Silver JB (2008) *Mosquito Ecology: Field sampling methods*. 3 edn. Springer, Netherlands.
- Simo FBN, Bigna JJ, Kenmoe S, Ndangang MS, Temfack E, Moundipa PF & Demanou M (2019) Dengue virus infection in people residing in Africa: a systematic review and meta-analysis of prevalence studies. *Sci Rep* 9: 13626. doi:10.1038/s41598-019-50135-x.
- Sinka ME (2013) global distribution of the dominant vector species of malaria: *Anopheles* mosquitoes-New insights into malaria vectors (ed. by S Manguin) InTech, Croatia, pp. 107-143.
- Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Okara RM, Van Boeckel T, Godfray HC, Harbach RE & Hay SI (2010) The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic precis. *Parasit Vectors* 3: 117. doi:10.1186/1756-3305-3-117.
- Six Sense (2021) *Earth Lab at Six Senses Ninh Van Bay runs the following sustainability initiatives*, Vol. 2022.
- Smith A & Carter ID (1984) International transportation of mosquitoes of public health importance: Commerce and the Spread of Pests and Disease Vectors (ed. by M Laird) Praeger, pp. 1-21.

- Smith DL & Ellis McKenzie F (2004) Statics and dynamics of malaria infection in *Anopheles* mosquitoes. *Malar J* 3: 13. doi:10.1186/1475-2875-3-13.
- Smith LB, Sears C, Sun H, Mertz RW, Kasai S & Scott JG (2019) CYP-mediated resistance and cross-resistance to pyrethroids and organophosphates in *Aedes aegypti* in the presence and absence of kdr. *Pesticide Biochemistry and Physiology* 160: 119-126. doi:<https://doi.org/10.1016/j.pestbp.2019.07.011>.
- Snow WF & Boreham PF (1979) The feeding habits and ecology of the tsetse fly *Glossina morsitans submorsitans* Newstead in relation to nagana transmission in the Gambia. *Acta Trop* 36: 47-51.
- Snyman LP (2021) Review of mosquitoes (Diptera: Culicidae) from the Afrotropical region using herpetofauna as hosts: conservation and epidemiological consequences of knowing little about many species. *Austral Entomology* 60: 163-171. doi:<https://doi.org/10.1111/aen.12501>.
- Socolovschi C, Pages F, Ndiath MO, Ratmanov P & Raoult D (2012) *Rickettsia* Species in African *Anopheles* Mosquitoes. *Plos one* 7: e48254. doi:10.1371/journal.pone.0048254.
- Soghigian J, Gloria-Soria A, Robert V, Le Goff G, Failloux A-B & Powell JR (2020) Genetic evidence for the origin of *Aedes aegypti*, the yellow fever mosquito, in the southwestern Indian Ocean. *Mol Ecol* 29: 3593-3606. doi:<https://doi.org/10.1111/mec.15590>.
- Sombie A, Saiki E, Yameogo F, Sakurai T, Shirozu T, Fukumoto S, Sanon A, Weetman D, McCall PJ, Kanuka H & Badolo A (2019) High frequencies of F1534C and V1016I kdr mutations and association with pyrethroid resistance in *Aedes aegypti* from Somgande (Ouagadougou), Burkina Faso. *Trop Med Health* 47: 2. doi:10.1186/s41182-018-0134-5.
- Sougoufara S, Diedhiou SM, Doucoure S, Diagne N, Sembene PM, Harry M, Trape JF, Sokhna C & Ndiath MO (2014) Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. *Malar J* 13: 125. doi:10.1186/1475-2875-13-125.

- South A, Lees R, Garrod G, Carson J, Malone D & Hastings I (2020) The role of windows of selection and windows of dominance in the evolution of insecticide resistance in human disease vectors. *Evol Appl* 13: 738-751. doi:<https://doi.org/10.1111/eva.12897>.
- Srisawat R, Komalamisra N, Eshita Y, Zheng M, Ono K, Itoh TQ, Matsumoto A, Petmitr S & Rongsriyam Y (2010) Point mutations in domain II of the voltage-gated sodium channel gene in deltamethrin-resistant *Aedes aegypti* (Diptera: Culicidae). *Applied Entomology and Zoology* 45: 275-282. doi:10.1303/aez.2010.275.
- Stenhouse SA, Plernsub S, Yanola J, Lumjuan N, Dantrakool A, Choochote W & Somboon P (2013) Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* (Diptera: Culicidae) by allele-specific PCR assay, and its distribution and effect on deltamethrin resistance in Thailand. *Parasit Vectors* 6: 253. doi:10.1186/1756-3305-6-253.
- Stevens PA (1984) Environmental management activities in malaria control in Africa. *Bull Wld Hlth Org* 62: 77-80.
- Stone CM & Foster WA (2013) Plant-sugar feeding and vectorial capacity: Ecology of parasite-vector interactions (ed. by W Takken & CJM Koenraadt) Wageningen Academic Publisher, Netherland.
- Stoney CM & Johnson LL (2018) Chapter 18 - Design of Clinical Trials and Studies: Principles and Practice of Clinical Research (Fourth Edition) (ed. by JI Gallin, FP Ognibene & LL Johnson) Academic Press, Boston, pp. 249-268.
- Stopard IJ, Churcher TS & Lambert B (2021) Estimating the extrinsic incubation period of malaria using a mechanistic model of sporogony. *PLoS Comput Biol* 17: e1008658. doi:10.1371/journal.pcbi.1008658.
- Subra R (1981) Biology and Control of *Culex pipiens quinquefasciatus* Say, 1823 (Diptera:Culicidae) with Special Reference to Africa. *Insect Sci. Appl.* 1: 319-338.
- Sutherst RW (2004) Global change and human vulnerability to vector-borne diseases. *Clin Microbiol Rev* 17: 136-173. doi:10.1128/cmr.17.1.136-173.2004.

- Takken W & Lindsay S (2019) Increased Threat of Urban Malaria from *Anopheles stephensi* Mosquitoes, Africa. *Emerg Infect Dis* 25: 1431-1433. doi:10.3201/eid2507.190301.
- Takken W, Snellen WB, Verhave JP & Knols BG (1990) Environmental measures for malaria control in Indonesia: an historical review on species sanitation. Wageningen Agricultural University Papers.
- Takken W & Verhulst NO (2013) Host preferences of blood-feeding mosquitoes. *Annu Rev Entomol* 58: 433-453. doi:10.1146/annurev-ento-120811-153618.
- Talipouo A, Mavridis K, Nchoutpouen E, Djappi-Tchamen B, Fotakis EA, Kopya E, Bamou R, Kekeunou S, Awono-Ambene P, Balabanidou V, Balaska S, Wondji CS, Vontas J & Antonio-Nkondjio C (2021) High insecticide resistance mediated by different mechanisms in *Culex quinquefasciatus* populations from the city of Yaounde, Cameroon. *Sci Rep* 11: 7322. doi:10.1038/s41598-021-86850-7.
- Tantely LM, Boyer S & Fontenille D (2015) A review of mosquitoes associated with Rift Valley fever virus in Madagascar. *Am J Trop Med Hyg* 92: 722-729. doi:10.4269/ajtmh.14-0421.
- Taylor B (1975a) Changes in the feeding behaviour of a malaria vector, *Anopheles farauti* Lav., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. *Trans R Ent Soc Lond* 127: 277-292.
- Taylor B (1975b) Changes in the feeding behaviour of a malaria vector, *Anopheles farauti* Lav., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. *Trans R Ent Soc Lond* 127: 277-292.
- Tedjou AN, Kamgang B, Yougang AP, Wilson-Bahun TA, Njiokou F & Wondji CS (2020) Patterns of Ecological Adaptation of *Aedes aegypti* and *Aedes albopictus* and *Stegomyia* Indices Highlight the Potential Risk of Arbovirus Transmission in Yaoundé, the Capital City of Cameroon. *Pathogens* 9: 491.
- Tetreau G, Stalinski R, David JP & Després L (2013) Monitoring resistance to *Bacillus thuringiensis* subsp. *israelensis* in the field by performing bioassays with each Cry toxin separately. *Mem Inst Oswaldo Cruz* 108: 894-900. doi:10.1590/0074-0276130155.

- The Revolutionary Government of Zanzibar (2015) Zanzibar Research Agenda 2015 - 2020: TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY, Zanzibar.
- The Revolutionary Government of Zanzibar (2020) Zanzibar Blue Economy Policy. Zanzibar Planning Commission.
- The World Tourism Organization (UNWTO) (2022) Sustainable development, Vol. 2022.
- Thornton J, Gomes B, Ayres C & Reimer L (2020) Insecticide resistance selection and reversal in two strains of *Aedes aegypti*. Wellcome Open Res 5: 183. doi:10.12688/wellcomeopenres.15974.2.
- Tikhonov G, Opedal ØH, Abrego N, Lehikoinen A, de Jonge MMJ, Oksanen J & Ovaskainen O (2020) Joint species distribution modelling with the r-package Hmsc. *Methods Ecol Evol* 11: 442-447. doi:<https://doi.org/10.1111/2041-210X.13345>.
- Tirados I, Costantini C, Gibson G & Torr SJ (2006) Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Med Vet Entomol* 20: 425-437. doi:10.1111/j.1365-2915.2006.652.x.
- Toledo ME, Rodriguez A, Valdes L, Carrion R, Cabrera G, Banderas D, Ceballos E, Domecq M, Pena C, Baly A, Vanlerberghe V & Van der Stuyft P (2011) Evidence on impact of community-based environmental management on dengue transmission in Santiago de Cuba. *Trop Med Int Health* 16: 744-747. doi:10.1111/j.1365-3156.2011.02762.x.
- Toto JC, Abaga S, Carnevale P & Simard F (2003) First report of the oriental mosquito *Aedes albopictus* on the West African island of Bioko, Equatorial Guinea. *Med Vet Entomol* 17: 343-346. doi:10.1046/j.1365-2915.2003.00447.x.
- Trewin BJ, Darbro JM, Jansen CC, Schellhorn NA, Zalucki MP, Hurst TP & Devine GJ (2017a) The elimination of the dengue vector, *Aedes aegypti*, from Brisbane, Australia: The role of surveillance, larval habitat removal and policy. *PLoS Negl Trop Dis* 11: e0005848-e0005848. doi:10.1371/journal.pntd.0005848.
- Trewin BJ, Darbro JM, Jansen CC, Schellhorn NA, Zalucki MP, Hurst TP & Devine GJ (2017b) The elimination of the dengue vector, *Aedes aegypti*, from Brisbane, Australia:

The role of surveillance, larval habitat removal and policy. PLoS Negl Trop Dis 11: e0005848. doi:10.1371/journal.pntd.0005848.

Tropical Pesticides Research Institute (TPRI) (2020) List of Registered Pesticides to be used in the United Republic of Tanzania 2020, Vol. 2021: TROPICAL PESTICIDES RESEARCH INSTITUTE Arusha - Tanzania.

Tuck SL, Phillips HR, Hintzen RE, Scharlemann JP, Purvis A & Hudson LN (2014) MODISTools - downloading and processing MODIS remotely sensed data in R. Ecol Evol 4: 4658-4668. doi:10.1002/ece3.1273.

Tun-Lin W, Burkot TR & Kay BH (2000) Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Med Vet Entomol 14: 31-37. doi:<https://doi.org/10.1046/j.1365-2915.2000.00207.x>.

Tun-Lin W, Kay BH & Burkot TR (1994a) Quantitative sampling of immature *Aedes aegypti* in metal drums using sweep net and dipping methods. J Am Mosq Control Assoc 10: 390 -390.

Tun-Lin W, Kay BH & Burkot TR (1994b) Quantitative sampling of immature *Aedes aegypti* in metal drums using sweep net and dipping methods. J Am Mosq Control Assoc 10: 390 -390.

Tuno N, Kjaerandsen J, Badu K & Kruppa T (2010) Blood-feeding behavior of *Anopheles gambiae* and *Anopheles melas* in Ghana, western Africa. J Med Entomol 47: 28-31.

Tuten HC, Bridges WC, Paul KS & Adler PH (2012) Blood-feeding ecology of mosquitoes in zoos. Med Vet Entomol 26: 407-416. doi:<https://doi.org/10.1111/j.1365-2915.2012.01012.x>.

U.S. President's Malaria Initiative Tanzania (Zanzibar) (2020) Malaria Operational Plan FY 2020.

UNICEF (2018) Assessment of the Impact of tourism on communities and children in Zanzibar, Zanzibar, United Republic of Tanzania.

- United Nation Development Programme (2017) A Socio-economic Impact Assessment of the Zika Virus in Latin America and the Caribbean: with a focus on Brazil, Colombia and Suriname: United Nation Development Programme, United States of America.
- Utzinger J, Tozan Y & Singer BH (2001) Efficacy and cost-effectiveness of environmental management for malaria control. *Trop Med Int Health* 6: 677-687. doi:10.1046/j.1365-3156.2001.00769.x.
- Vairo F, Nicastrì E, Meschi S, Schepisi MS, Paglia MG, Bevilacqua N, Mangi S, Sciarrone MR, Chiappini R, Mohamed J, Racalbuto V, Di Caro A, Capobianchi MR & Ippolito G (2012) Seroprevalence of dengue infection: a cross-sectional survey in mainland Tanzania and on Pemba Island, Zanzibar. *Int J Infect Dis* 16: e44-46. doi:10.1016/j.ijid.2011.09.018.
- Vairo F, Nicastrì E, Yussuf SM, Cannas A, Meschi S, Mahmoud MA, Mohamed AH, Maiko PM, De Nardo P, Bevilacqua N, Castilletti C, Di Caro A, Racalbuto V & Ippolito G (2014) IgG against dengue virus in healthy blood donors, Zanzibar, Tanzania. *Emerg Infect Dis* 20: 465-468. doi:10.3201/eid2003.130150.
- van Emden HF & Service MW (2004) *Pest and Vector Control*. Cambridge University Press, United Kingdom.
- Vanlerberghe V, Toledo ME, Rodriguez M, Gomez D, Baly A, Benitez JR & Van der Stuyft P (2010) Community involvement in dengue vector control: cluster randomised trial. *MEDICC Rev* 12: 41-47.
- Vázquez DP & Aizen MA (2004) Asymmetric specialization: A pervasive feature of plant-pollinator interactions. *Ecology* 85: 1251 - 1257.
- Vázquez DP, Melián CJ, Williams NM, Blüthgen N, Krasnov BR & Poulin R (2007) Species abundance and asymmetric interaction strength in ecological networks. *Oikos* 116: 1120-1127.
- Verdonschot PFM & Besse-Lototskaya AA (2014) Flight distance of mosquitoes (Culicidae): A metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. *Limnologia* 45: 69-79. doi:<https://doi.org/10.1016/j.limno.2013.11.002>.

- Villanueva-Segura K, Ponce-Garcia G, Lopez-Monroy B, Mora-Jasso E, Perales L, Gonzalez-Santillan FJ, Ontiveros-Zapata K, Davila-Barboza JA & Flores AE (2020) Multiplex PCR for simultaneous genotyping of kdr mutations V410L, V1016I and F1534C in *Aedes aegypti* (L.). *Parasit Vectors* 13: 325. doi:10.1186/s13071-020-04193-0.
- Vinson JE, Gottdenker NL, Chaves LF, Kaul RB, Kramer AM, Drake JM & Hall RJ (2022) Land reversion and zoonotic spillover risk. *Royal Society Open* 9.
- Vittor AY, Pan W, Gilman RH, Tielsch J, Glass G, Shields T, Sanchez-Lozano W, Pinedo VV, Salas-Cobos E, Flores S & Patz JA (2009) Linking deforestation to malaria in the Amazon: characterization of the breeding habitat of the principal malaria vector, *Anopheles darlingi*. *Am J Trop Med Hyg* 81: 5-12.
- Wada Y, Kawai S, Ito S, Oda T, Nishigaki J, Suenaga O & Omori N (1970) Ecology of Vector Mosquitoes of Japanese Encephalitis, Especially of *Culex tritaeniorhynchus*. 2. Nocturnal activity and host preference based on all-night-catches by different methods in 1965 and 1966 near Nagasaki City.\*. *Trop Medicine* 12: 79-89.
- Walker M, Robert MA & Childs LM (2021) The importance of density dependence in juvenile mosquito development and survival: A model-based investigation. *Ecological Modelling* 440: 109357. doi:<https://doi.org/10.1016/j.ecolmodel.2020.109357>.
- Walshe DP, Garner P, Abdel-Hameed Adeel AA, Pyke GH & Burkot T (2013) Larvivorous fish for preventing malaria transmission. *Cochrane Database Syst Rev*: CD008090. doi:10.1002/14651858.CD008090.pub2.
- Walter Reed Biosystematics Unit (2021) Mosquito Occurrence Dataset: by WRB Unit) Smithsonian Institution.
- Wang Y, Naumann U, Wright ST & Warton DI (2012) mvabund – an R package for model-based analysis of multivariate abundance data. *Methods Ecol and Evol* 3: 471-474.
- Ward T, Samuel M, Maoz D, Runge-Ranzinger S, Boyce R, Toledo J, Velayudhan R & Horstick O (2017) Dengue data and surveillance in Tanzania: a systematic literature review. *Trop Med Int Health* 22: 960-970. doi:10.1111/tmi.12903.

- Warton DI, Blanchet FG, O'Hara RB, Ovaskainen O, Taskinen S, Walker SC & Hui FKC (2015) So Many Variables: Joint Modeling in Community Ecology. *Trends Ecol Evol* 30: 766-779. doi:<https://doi.org/10.1016/j.tree.2015.09.007>.
- Water Sanitation Hygiene and Health (2017) Pesticides used in water for public health purposes: Guidelines for Drinking-Water Quality (ed. by WH Organization) World Health Organization, Geneva, pp. 190-200.
- Watson M (1911) The prevention of malaria in the Federated Malay States. The Liverpool School of Tropical Medicine, Liverpool, UK.
- Weitz B (1960) Feeding habits of bloodsucking arthropods. *Experimental Parasitology* 9: 63-82.
- Whalon ME, Mota-Sanchez D & Hollingworth RM (2008) Analysis of Global Pesticide Resistance in Arthropods: Global Pesticide Resistance in Arthropods (ed. by ME Wholan, D Mota-Sanchez & RM Hollingworth) CAB International, United Kingdom, pp. 5-31.
- Whelan PI, Kurucz N, Pettit WJ & Krause V (2020) Elimination of *Aedes aegypti* in northern Australia, 2004-2006. *J Vector Ecol* 45: 118-126. doi:10.1111/jvec.12379.
- White BG (1989) Lymphatic filariasis: Geographical Distribution of Arthropod-borne Diseases and Their Principal Vectors (ed. World Health Organization, Geneva, pp. 7-23.
- White BG & Moore SJ (2015) Terminology of Insect Repellents: Insect Repellents Handbook (ed. by M Debboun, SP Frances & DA Strickman) CRC Press, pp. 3-30.
- White GB (1974) *Anopheles gambiae* complex and disease transmission in Africa. *Trans R Soc Trop Med Hyg* 68: 278-301.
- White GB (1985) *Anopheles bwambiae* sp.n., a malaria vector in the Semliki Valley, Uganda, and its relationships with other sibling species of the *An.gambiae* complex (Diptera: Culicidae). *Systematica Entomol* 10: 501-522.
- WHO (1975) Manual on practical entomology in malaria. Part II: Methods and Techniques. World Health Organization, Geneva.

- WHO (1982) Manual on environmental management for mosquito control with special emphasis on malaria vectors. World Health Organization, Geneva, Switzerland.
- WHO (2006) Pesticides and their application for the control of vectors and pests of public health importance. 6 edn. WHO, Geneva.
- WHO (2012) Handbook for integrated vector management. WHO Library Cataloguing-in-Publication Data, Geneva, Switzerland.
- WHO (2013) Larval source management: A supplementary measure for malaria vector control (An operational manual). World Health Organization, Geneva, Switzerland.
- WHO (2014) A global brief on vector-borne diseases: World Health Organization, p. 56.
- WHO (2016) Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticide on treated surfaces: WHO, Geneva, Switzerland.
- WHO (2017a) Global vector control response 2017-2030: World Health Organization, Geneva, Switzerland, p. 53.
- WHO (2017b) How to design vector control efficacy trials, guidance on phase III vector control field design: World Health Organization.
- WHO (2018) Japanese encephalitis: Fact sheet, Vol. 2022: World Health Organization.
- WHO (2019a) Weekly bulletin on outbreaks and other emergencies.
- WHO (2019b) Zika Epidemiology Report: Global overview.
- WHO (2020a) Vector-borne diseases: WHO fact sheet, Vol. 2022: World Health Organization.
- WHO (2020b) World malaria report 2019: World Health Organization, Geneva, Switzerland, p. 196.
- WHO (2021) World malaria report 2020: World Health Organization, Geneva, Switzerland, p. 196.
- WHO (2022) Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. World Health Organization (WHO).

- Wilder-Smith A, Gubler DJ, Weaver SC, Monath TP, Heymann DL & Scott TW (2017) Epidemic arboviral diseases: priorities for research and public health. *Lancet Infect Dis* 17: 101-106.
- Wilkerson RC, Linton Y-M, Fonseca DM, Schultz TR, Price DC & Strickman DA (2015) Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini that Balances Utility with Current Knowledge of Evolutionary Relationships. *Plos one* 10: e0133602.
- Wilkerson RC, Linton Y-M & Stickman D (2021) Mosquitoes of the world. John Hopkins University Press.
- Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ & Lindsay SW (2020) The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Negl Trop Dis* 14: e0007831. doi:10.1371/journal.pntd.0007831.
- Worrall E & Fillinger U (2011) Large-scale use of mosquito larval source management for malaria control in Africa: a cost analysis. *Malar J* 10: 338. doi:10.1186/1475-2875-10-338.
- WTTC (2021) Global economic impact, Vol. 2022: World Travel and Tourism Council.
- Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, Gandhe SS, Gokhle MD, Jacob GP, Hundekar SL & Mishra AC (2006) Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 12: 1580-1583. doi:10.3201/eid1210.060529.
- Yoshikawa MJ, Kusriastuti R & Liew C (2020) An interdisciplinary study: disseminating information on dengue prevention and control in the world-famous travel destination, Bali, Indonesia. *Evolutionary and Institutional Economics Review* 17: 265-293. doi:10.1007/s40844-019-00138-0.
- Youngang AP, Kamgang B, Bahun TAW, Tedjou AN, Nguiffo-Nguete D, Njiokou F & Wondji CS (2020) First detection of F1534C knockdown resistance mutation in *Aedes aegypti* (Diptera: Culicidae) from Cameroon. *Infectious Diseases of Poverty* 9: 152. doi:10.1186/s40249-020-00769-1.

- Zahouli JBZ, Koudou BG, Muller P, Malone D, Tano Y & Utzinger J (2017) Urbanization is a main driver for the larval ecology of Aedes mosquitoes in arbovirus-endemic settings in south-eastern Cote d'Ivoire. PLoS Negl Trop Dis 11: e0005751. doi:10.1371/journal.pntd.0005751.
- Zanzibar Association of Tourism Investors (ZATI) (2017) ZATI Statistical Analysis of Tourism Arrivals to Zanzibar End-Year 2016, Zanzibar, Tanzania.
- Zanzibar Malaria Elimination Programme (2009) Malaria Elimination in Zanzibar: A Feasibility Assessment, p. 81.
- Zanzinet Forum (2019) Climate and Soils: Zanzinet.
- Zlotikin E (2001) Insecticides Affecting Voltage-Gated Ion Channels: Biochemical Sites of Insecticide Action and Resistance (ed. by I Ishaaya) Springer, pp. 43-69.
- Zuharah WF & Sufian M (2021) The discovery of a novel knockdown resistance (kdr) mutation A1007G on Aedes aegypti (Diptera: Culicidae) from Malaysia. Sci Rep 11: 5180. doi:10.1038/s41598-021-84669-w.
- Zuur AF, Leno EN & Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol and Evol 1: 3-14.

## **APPENDIXES**

## APPENDIX CHAPTER 2

**Table A2.1.** Relative abundance (total/species/site) and composition of emerged adult mosquitoes obtained from larval collections.

| Genus        | Species                       | Hotel A |                         | Hotel B |                         | Hotel C |                         | Hotel D |                         |
|--------------|-------------------------------|---------|-------------------------|---------|-------------------------|---------|-------------------------|---------|-------------------------|
|              |                               | Total   | $d = (Ni/N) \times 100$ | Total   | $d = (Ni/N) \times 100$ | Total   | $d = (Ni/N) \times 100$ | Total   | $d = (Ni/N) \times 100$ |
| <i>Aedes</i> | <i>Aedes aegypti</i>          | 4900    | 75.0                    | 3169    | 79.7                    | 2862    | 81.8                    | 5276    | 63.5                    |
|              | <i>Aedes africanus</i>        | 0       | 0.0                     | 0       | 0.0                     | 0       | 0.0                     | 8       | 0.1                     |
|              | <i>Aedes bromeliae/lilii</i>  | 165     | 2.5                     | 13      | 0.3                     | 3       | 0.1                     | 1159    | 14.0                    |
|              | <i>Aedes calceatus</i>        | 191     | 2.9                     | 0       | 0.0                     | 0       | 0.0                     | 25      | 0.3                     |
|              | <i>Aedes heischi</i>          | 17      | 0.3                     | 0       | 0.0                     | 0       | 0.0                     | 0       | 0.0                     |
|              | <i>Aedes metallicus</i>       | 188     | 2.9                     | 17      | 0.4                     | 61      | 1.7                     | 688     | 8.3                     |
|              | <i>Aedes natalensis</i>       | 2       | 0.0                     | 0       | 0.0                     | 0       | 0.0                     | 1       | 0.0                     |
|              | <i>Aedes simpson</i>          | 49      | 0.8                     | 4       | 0.1                     | 2       | 0.1                     | 694     | 8.4                     |
|              | <i>Aedes unilineatus</i>      | 0       | 0.0                     | 0       | 0.0                     | 0       | 0.0                     | 3       | 0.0                     |
|              | <i>Aedes vittatus</i>         | 0       | 0.0                     | 0       | 0.0                     | 0       | 0.0                     | 36      | 0.4                     |
| <i>Culex</i> | <i>Culex cinerellus</i>       | 0       | 0.0                     | 16      | 0.4                     | 0       | 0.0                     | 0       | 0.0                     |
|              | <i>Culex cinereus</i>         | 6       | 0.1                     | 81      | 2.0                     | 7       | 0.2                     | 36      | 0.4                     |
|              | <i>Culex nebulosus</i>        | 0       | 0.0                     | 0       | 0.0                     | 0       | 0.0                     | 2       | 0.0                     |
|              | <i>Culex pipiens</i>          | 0       | 0.0                     | 0       | 0.0                     | 7       | 0.2                     | 0       | 0.0                     |
|              | <i>Culex quinquefasciatus</i> | 403     | 6.2                     | 547     | 13.8                    | 230     | 6.6                     | 120     | 1.4                     |

|                       |                                      |     |     |    |     |     |     |    |     |
|-----------------------|--------------------------------------|-----|-----|----|-----|-----|-----|----|-----|
|                       | <i>Culex tigripes</i>                | 35  | 0.5 | 7  | 0.2 | 0   | 0.0 | 46 | 0.6 |
| <i>Eretmapodites</i>  | <i>Eretmapodites chrysogaster</i>    | 201 | 3.1 | 1  | 0.0 | 0   | 0.0 | 0  | 0.0 |
|                       | <i>Eretmapodites conchobius</i>      | 0   | 0.0 | 0  | 0.0 | 0   | 0.0 | 16 | 0.2 |
|                       | <i>Eretmapodites inornatus</i>       | 46  | 0.7 | 4  | 0.1 | 10  | 0.3 | 0  | 0.0 |
|                       | <i>Eretmapodites quinquevittatus</i> | 200 | 3.1 | 81 | 2.0 | 296 | 8.5 | 82 | 1.0 |
|                       | <i>Eretmapodites subsimplicipes</i>  | 110 | 1.7 | 19 | 0.5 | 16  | 0.5 | 27 | 0.3 |
| <i>Anopheles</i>      | <i>Anopheles gambiae s.l</i>         | 0   | 0.0 | 13 | 0.3 | 0   | 0.0 | 0  | 0.0 |
| <i>Orthopodomyia</i>  | <i>Orthopodomyia spp</i>             | 6   | 0.1 | 0  | 0.0 | 0   | 0.0 | 0  | 0.0 |
| <i>Toxorhynchites</i> | <i>Toxorhynchites brevialpis</i>     | 14  | 0.2 | 2  | 0.1 | 3   | 0.1 | 84 | 1.0 |

**Table A2.2.** Relative abundance (total/species/site) and composition of adult mosquitoes obtained from combined CDC light trap and Prokopack catches.

| Genus                | Species                           | Hotel A |                  | Hotel B |                  | Hotel C |                  | Hotel D |                  |
|----------------------|-----------------------------------|---------|------------------|---------|------------------|---------|------------------|---------|------------------|
|                      |                                   | Total   | d = (Ni/N) * 100 | Total   | d = (Ni/N) * 100 | Total   | d = (Ni/N) * 100 | Total   | d = (Ni/N) * 100 |
| <i>Aedes</i>         | <i>Aedes aegypti aegypti</i>      | 0       | 0.0              | 9       | 4.6              | 14      | 1.6              | 69      | 31.2             |
|                      | <i>Aedes africanus</i>            | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Aedes bromeliae/lilii</i>      | 0       | 0.0              | 0       | 0.0              | 1       | 0.1              | 2       | 0.9              |
|                      | <i>Aedes calceatus</i>            | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Aedes heischi</i>              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Aedes simpsoni</i>             | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 12      | 5.4              |
|                      | <i>Aedes natalensis</i>           | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Aedes unilineatus</i>          | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Aedes vittatus</i>             | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
| <i>Culex</i>         | <i>Culex cinerellus</i>           | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Culex cinereus</i>             | 0       | 0.0              | 5       | 2.6              | 19      | 2.1              | 0       | 0.0              |
|                      | <i>Culex nebulosus</i>            | 3       | 3.8              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Culex pipiens</i>              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Culex quinquefasciatus</i>     | 75      | 96.2             | 181     | 92.3             | 833     | 93.0             | 135     | 61.1             |
|                      | <i>Culex tigripes</i>             | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Culex rima</i>                 | 0       | 0.0              | 0       | 0.0              | 25      | 2.8              | 0       | 0.0              |
|                      | <i>Culex tigripes</i>             | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Culex simpsoni</i>             | 0       | 0.0              | 0       | 0.0              | 1       | 0.1              | 0       | 0.0              |
| <i>Eretmapodites</i> | <i>Eretmapodites chrysogaster</i> | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |
|                      | <i>Eretmapodites conchobius</i>   | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              | 0       | 0.0              |

|                       |                                      |   |     |   |     |   |     |   |     |
|-----------------------|--------------------------------------|---|-----|---|-----|---|-----|---|-----|
|                       | <i>Eretmapodites inornatus</i>       | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
|                       | <i>Eretmapodites quinquevittatus</i> | 0 | 0.0 | 0 | 0.0 | 2 | 0.2 | 2 | 0.9 |
|                       | <i>Eretmapodites subsimplicipes</i>  | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 0.5 |
| <i>Anopheles</i>      | <i>Anopheles gambiae s.l</i>         | 0 | 0.0 | 1 | 0.5 | 0 | 0.0 | 0 | 0.0 |
| <i>Orthopodomyia</i>  | <i>Orthopodomyia spp</i>             | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| <i>Toxorhynchites</i> | <i>Toxorhynchites brevipalpis</i>    | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |

**Table A2.3.** Relative abundance (total/species/site) of mosquito species from combined larva (L) and adult (A) collections and reported type of confirmed and suspected associated human pathogens.

| Species                      | Hotel A | Hotel B | Hotel C | Hotel D | Associated human pathogen                                                              | Ref                                                                                                     |
|------------------------------|---------|---------|---------|---------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
|                              | L+A     | L+A     | L+A     | L+A     |                                                                                        |                                                                                                         |
| <i>Aedes aegypti</i>         | 4900    | 3169    | 2862    | 5276    | DENV, CHIKV, YFV, ZIKV, RVFV, BBKV, MYRV, USUV, Lymphatic filariasis (Pacific Islands) | Christophers (1960); McIntosh et al., (1973); Berger (1975); Turell et al., (2008); Long et al., (2011) |
| <i>Aedes africanus</i>       | 0       | 0       | 0       | 8       | DENV, YFV, WNV, RVF, and ZIKV*                                                         | Wilkerson et al. (2015)                                                                                 |
| <i>Aedes bromeliae/lilii</i> | 165     | 13      | 4       | 1159    | YFV and CHIKV*                                                                         | Berger (1975); Baudon et al., (1984)                                                                    |
| <i>Aedes calceatus</i>       | 191     | 0       | 0       | 25      | RVFV*                                                                                  | Wilkerson et al. (2015)                                                                                 |
| <i>Aedes heischi</i>         | 17      | 0       | 0       | 0       | Unknown vector status, Nuisance species                                                | Wilkerson et al. (2015)                                                                                 |
| <i>Aedes metallicus</i>      | 188     | 17      | 61      | 688     | YFV                                                                                    | Wilkerson et al. (2015)                                                                                 |
| <i>Aedes natalensis</i>      | 2       | 0       | 0       | 1       | Unknown vector status, Nuisance species                                                | Turell et al., (2008)                                                                                   |
| <i>Aedes simpsoni</i>        | 49      | 4       | 2       | 694     | BBKV, YFV                                                                              | Wilkerson et al. (2015)                                                                                 |
| <i>Aedes unilineatus</i>     | 0       | 0       | 0       | 3       | ZIKV*                                                                                  | Huang (1997)                                                                                            |

|                                            |     |     |     |     |                                                                     |                                                            |
|--------------------------------------------|-----|-----|-----|-----|---------------------------------------------------------------------|------------------------------------------------------------|
| <i>Aedes vittatus</i>                      | 0   | 0   | 0   | 36  | CHIKV, BBKV and ZIKV, NRIV, PGAV, SFV                               | McIntosh et al., (1973)                                    |
| <i>Culex cinerellus</i>                    | 0   | 16  | 0   | 0   | Unknown vector status, Nuisance species                             |                                                            |
| <i>Culex cinereus</i>                      | 6   | 86  | 26  | 36  | CHIKV, MIDV, BBKV                                                   | Berger (1975); Hartberg & Gerberg (1971)                   |
| <i>Culex nebulosus</i>                     | 3   | 0   | 0   | 2   | MIDV, BBKV, BGIV                                                    | Berger (1975); Hartberg & Gerberg (1971)                   |
| <i>Culex pipiens</i>                       | 0   | 0   | 7   | 0   | JEV, LACV, SFV, SLEV, WEEV, WNV, BANV, BUNV, USUV                   | Berger (1975); Hartberg & Gerberg (1971)                   |
| <i>Culex quinquefasciatus</i>              | 478 | 728 | 963 | 120 | CHIKV, EEEV, MURV, RRV, SLEV, SINV, WNV, BBKV, Lymphatic filariasis | Berger (1975); (Hartberg & Gerberg, (1971)                 |
| <i>Culex rima</i>                          | 0   | 0   | 25  | 0   | BANV                                                                | Berger (1975)                                              |
| <i>Culex tigripes</i>                      | 35  | 7   | 0   | 0   | SINV, BBKV                                                          | Mulwa et al., (2018); (Berger, 1975); (Mulwa et al., 2018) |
| <i>Culex simpsoni</i>                      | 0   | 0   | 1   | 0   | Unknown vector status, Nuisance species                             |                                                            |
| <i>Eretmapodites chrysogaster</i>          | 201 | 1   | 0   | 0   | YFV*                                                                | Berger, (1975)                                             |
| <i>Eretmapodites silvestris conchobius</i> | 0   | 0   | 0   | 0   | Unknown vector status, Nuisance species                             |                                                            |
| <i>Eretmapodites inornatus</i>             | 46  | 4   | 10  | 0   | Unknown vector status, Nuisance species                             |                                                            |

|                                      |     |    |     |    |                                                          |               |
|--------------------------------------|-----|----|-----|----|----------------------------------------------------------|---------------|
| <i>Eretmapodites quinquevittatus</i> | 200 | 81 | 298 | 82 | YFV*                                                     | Berger (1975) |
| <i>Eretmapodites subsimplicipes</i>  | 110 | 19 | 0   | 27 | Unknown vector status, Nuisance species                  |               |
| <i>Anopheles gambiae s.l</i>         | 0   | 14 | 0   | 0  | Human <i>Plasmodium spp</i> , Lymphatic filariaisi, ONNV | Berger (1975) |
| <i>Orthopodomyia spp</i>             | 6   | 0  | 0   | 0  | Possible vectors of arboviruses among birds and mammals  |               |
| <i>Toxorhynchites brevipalpis</i>    | 14  | 2  | 0   | 0  | No vector                                                |               |

BANZV = Banzi virus, BBKV = Babanki virus, BGIV = Bangui virus, BUNV =Bunyamwera virus, DENV = Dengue virus, CHIKV=Chikungunya, EEEV = Eastern Equine Encephalitis virus, JEV = Japanese Encephalitis virus, LACV = LaCrosse Encephalitis virus, MURV = Murray Valley virus, MIDV = Middelburg virus, MYRV = Mayaro virus, NRIV=Ngari virus, ONNV = O’Nyong-Nyong virus, PGAV=Pongola virus, RVFV = Rift Valley Fever Virus (RVFV), SLEV = St. Louis Encephalitis virus, SPONV =Spondweni virus, SINV = Sindbis virus, RVF =Rift valley Fever virus, RRV=Ross River virus, SINV = Sindbis virus, SFV = Semliki Forest virus, USUV = Usutu virus, YFV= Yellow Fever virus, ZIKV= Zika virus. \*Experimental infection

## APPENDIX: CHAPTER 4

**Table A4.1.** Copy of questionnaire on existing vector control practices and chemical insecticide use at hotels Zanzibar Island

State University of Zanzibar/University of Copenhagen/University of Pretoria  
Existing Mosquito Control Practices at Hotels, EnSuZa Project WP 2)

**Hotel ID:**

**Date:**

**Name of interviewer:**

**Name of respondent:**

**Current position of interviewer:**

**Current position of respondent:**

**Contact details:**

**Contact details:**

**Signature:**

**Signature:**

### Indoor Areas

Please indicate the type of mosquito control and where this is currently applied on a routine basis (✓)

| Type of control | Guest rooms | Hall/Lounge/<br>Foyer | Restaurant | Spa/Fitness | Kitchen | Bathroom/<br>Toilet | Offices | Staff quarters | Other: |
|-----------------|-------------|-----------------------|------------|-------------|---------|---------------------|---------|----------------|--------|
| Repellent       |             |                       |            |             |         |                     |         |                |        |
| Space spray     |             |                       |            |             |         |                     |         |                |        |

|                         |  |  |  |  |  |  |  |  |  |
|-------------------------|--|--|--|--|--|--|--|--|--|
| Fumigant/fogging        |  |  |  |  |  |  |  |  |  |
| Bed nets                |  |  |  |  |  |  |  |  |  |
| Window/door screens     |  |  |  |  |  |  |  |  |  |
| Breeding site removal   |  |  |  |  |  |  |  |  |  |
| Breeding site treatment |  |  |  |  |  |  |  |  |  |
| Other:                  |  |  |  |  |  |  |  |  |  |
| Other:                  |  |  |  |  |  |  |  |  |  |
| Other:                  |  |  |  |  |  |  |  |  |  |
| Other:                  |  |  |  |  |  |  |  |  |  |

Further comments and observations: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

*(continues next page)*

**Hotel ID:**

**Date:**

### Indoor Areas

Please indicate for all mosquito control types, their name, how often, how much, at what cost and by whom they are applied (✓ or text)

| Type of control         | Technical or Commercial name(s) | Application Interval: |        |         |                  |             |          | Quantity per time unit e.g., litres/month | Cost per time unit e.g., \$/month | Applied by        |               |                     |        |
|-------------------------|---------------------------------|-----------------------|--------|---------|------------------|-------------|----------|-------------------------------------------|-----------------------------------|-------------------|---------------|---------------------|--------|
|                         |                                 | Daily                 | Weekly | Monthly | Quarter annually | Bi-annually | Annually |                                           |                                   | Maintenance Staff | House keepers | External personnel* | Other: |
| Repellent               |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Space spray             |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Fumigant/fogging        |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Fumigant/fogging        |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Bed nets                |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Window/door screen      |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Breeding site removal   |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Breeding site treatment |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Other:                  |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Other:                  |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |
| Other:                  |                                 |                       |        |         |                  |             |          |                                           |                                   |                   |               |                     |        |

\*Please, note name of company and country of origin: Monthly fumigation has been carried out by PestGuard, a company from Dar-es-Salaam

Further comments and observations: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Hotel ID:**

**Date:**

*(Continues next page)*

## Outdoor Areas

Please indicate for all mosquito control types, their name, how often, how much, at what cost and by whom they are applied (✓ or text)

| Type of control         | Technical or Commercial name(s) | Application Interval: |        |         |                  |             |          | Quantity per time unit e.g. litres/month | Cost per time unit e.g. \$/month | Applied by        |              |                               |        |
|-------------------------|---------------------------------|-----------------------|--------|---------|------------------|-------------|----------|------------------------------------------|----------------------------------|-------------------|--------------|-------------------------------|--------|
|                         |                                 | Daily                 | Weekly | Monthly | Quarter annually | Bi-annually | Annually |                                          |                                  | Maintenance Staff | House keeper | External personnel /company * | Other: |
| Repellent               |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Space spray             |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Fumigant/fogging        |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Fumigant/fogging        |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Bed nets                |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Window/door screen      |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Breeding site removal   |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Breeding site treatment |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Other:                  |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Other:                  |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |
| Other:                  |                                 |                       |        |         |                  |             |          |                                          |                                  |                   |              |                               |        |

\*Please, note name of company and country of origin:

Further comments and observations: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Hotel ID:**

**Date:**

### **Outdoor Areas**

Please encircle directly on map the areas and the type(s) of mosquito control currently used on a routine basis (using given abbreviation)

#### **Type of control**

Space spray: **SS**

Fumigant/fogging: **F**

Breeding site removal: **BSR**

Breeding site treatment: **BST**

Spatial Repellent: **SR**

Bed net: **B**

Window/door screen: **WS**

Other:

**Satellite image of the hotel**

**Table A4.2:** Primers used for allele-specific qPCR of the VGSC (A) and CNV assay of the GSTe2 gene (B)

| <b>A)</b>         |                              |                     |                                   |
|-------------------|------------------------------|---------------------|-----------------------------------|
| <b>Primer</b>     | <b>Sequence (5' - 3')</b>    | <b>Product Size</b> | <b>Ref.</b>                       |
| F1534 (for WT)    | #TCTACTTTGTGTTCTTCATCATATT   | 90 bp               | Linss et al., (2014)              |
| 1534 (common rev) | TCTGCTCGTTGAAGTTGTCGAT       |                     |                                   |
| 1534C (for MT)    | ##TCTACTTTGTGTTCTTCATCATGTG  | 110 bp              | Linss et al., (2014)              |
| 1534 (common rev) | TCTGCTCGTTGAAGTTGTCGAT       |                     |                                   |
| V1016 (for WT)    | ##ACAAATTGTTTCCCACCCGCACCGG  | 102 bp              |                                   |
| 1016 (common rev) | GGATGAACCGAAATTGGACAAAAGC    |                     |                                   |
| 1016I (for MT)    | #ACAAATTGTTTCCCACCCGCACTG    | 82 bp               | Saavedra-Rodriguez et al., (2018) |
| 1016 (common rev) | GGATGAACCGAAATTGGACAAAAGC    |                     |                                   |
| V1016 (rev WT)    | ##CTCTACTTTGTGTTCTTCATCATGTG | 80 bp               | Saavedra-Rodriguez et al., (2007) |
| 1016 (common for) | TCTGCTCGTTGAAGTTGTCGAT       |                     |                                   |
| 1016G (rev MT)    | #CAGCAAGGCTAAGAAAAGGTAACTC   | 60 bp               |                                   |
| 1016 (common for) | TCTGCTCGTTGAAGTTGTCGAT       |                     |                                   |
| V410 (for WT)     | ##ATCTTCTTGGGTTCGTTCTACCGTG  | 180 bp              | Stenhouse et al., (2013)          |
| 410 (common rev)  | TTCTTCCTCGGCGGCCTCTT         |                     |                                   |
| 410L (for MT)     | #ATCTTCTTGGGTTCGTTCTACCATT   | 160 bp              | Stenhouse et al., (2013)          |
| 410 (common rev)  | TTCTTCCTCGGCGGCCTCTT         |                     |                                   |
| Long 5' - tail    | GCGGGCAGGGCGGCGGGGGCGGGGCC   | 25 bp               |                                   |
| Short 5' - tail   | GCGGGC                       | 5 bp                |                                   |
| <b>B)</b>         |                              |                     |                                   |
| GSTE2CNV (for)    | GCGGATTTCAGTTGCGTTTCG        | 149 bp              | This study                        |
| GSTE2CNV (rev)    | ACCTGCTCGGCTCCACTTC          |                     |                                   |

#: short 5' - tail attached, ##: long 5' - tail attached

**Table A4.3:** Primers used to amplify and sequence partial segment 6 in domain I-IV (A) and whole GSTe2 gene (B)

| A)              |                             |                                              |              |                                             |
|-----------------|-----------------------------|----------------------------------------------|--------------|---------------------------------------------|
| Primer          | Sequence 5'-3'              | Target region                                | Product size | Ref.                                        |
| <b>DomI_F*+</b> | GATAATCCAAATTACGG<br>GTATAC | Dom I,<br>Seg. 6,<br>Exon 9<br>and 10        | ~500 bp      | Saavedra-<br>Rodriguez<br>et al.,<br>(2018) |
| <b>DomI_R*+</b> | TTCTTCCTCGGCGGCCTC<br>TT    | Dom I,<br>Seg. 6<br><br>Exon 9<br>and 10     | ~500 bp      | Saavedra-<br>Rodriguez<br>et al.,<br>(2018) |
| <b>AaSCF20*</b> | GACAATGTGGATCGCTT<br>CCC    | Dom II,<br>Seg. 6,<br>Exon 20<br>and 21      | ~630 bp      |                                             |
| <b>AaSCR21*</b> | GCAATCTGGCTTGTT<br>AACTTG   | Dom II,<br>Seg. 6,<br>Exon 20<br>and 21      | ~630 bp      |                                             |
| <b>AaSCF3+</b>  | GTGGAACCTTCACCGACTT<br>CA   | Dom II<br>Seg. 6<br><br>Exon 20<br>and 21    | NA           | Chung et<br>al., (2019)                     |
| <b>AaSCR22+</b> | TTCACGAACTTGAGCGC<br>GTTG   | Dom II<br>Seg. 6<br><br>Exon 20<br>and 21    | NA           | Chung et<br>al., (2019)                     |
| <b>AaSCF7*+</b> | GAGAACTCGCCGATGAA<br>CTT    | Dom III,<br>Seg. 6,<br>Exon 30,<br>31 and 32 | ~748 bp      | Chung et<br>al., (2019)                     |
| <b>AaSCR7*</b>  | GACGACGAAATCGAACA<br>GGT    | Dom III,<br>Seg. 6,<br>Exon 30,<br>31 and 32 | ~748 bp      |                                             |

|                |                          |                                                      |         |                         |
|----------------|--------------------------|------------------------------------------------------|---------|-------------------------|
| <b>AaSCR8+</b> | TAGCTTTCGCGGCTTCTT<br>C  | Dom III,<br>Seg. 6,<br>Exon 30,<br>31 and 32         | NA      |                         |
| <b>AlSCF6*</b> | TCGAGAAGTACTTCGTGT<br>CG | Dom IV,<br>P-region<br>connecting<br>seg. 5 and<br>6 | ~280 bp | Chung et<br>al., (2019) |

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**B)**

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|                            |                             |               |       |            |
|----------------------------|-----------------------------|---------------|-------|------------|
| <b>GSTE2_Amp_Forward*</b>  | CCCCGCTCGTACTGTCTA<br>T     | GSTe2<br>gene | ~1760 | This study |
| <b>GSTE2_Amp_Reverse*</b>  | CCATGAAGCGATTCTCA<br>ATCCTA | GSTe2<br>gene | ~1760 | This study |
| <b>GSTE2_Seq1_Forward+</b> | CTCGTTTGCGGTTTGTGT<br>GT    | GSTe2<br>gene | NA    | This study |
| <b>GSTE2_Seq2_Forward+</b> | GAGGGTCTGAGATTCCA<br>GCG    | GSTe2<br>gene | NA    | This study |
| <b>GSTE2_Seq3_Foward+</b>  | GCAAACGAACCGGTCAC<br>AAA    | GSTe2<br>gene | NA    | This study |

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\*: Amplifying primer, +: Sequencing primer

110 120 130 140 150 160 170 180 190 200  
 LOC110676855 RLRFVCEPILFAGGSEIPADRAEYVQKAYQLLEDTLVDDYIVGNSLTIADFSCVSVSSVIMGVIPMDKEKFPKRIYQWLDRLKALPYEAA  
 Haplotype 1 .S..F.....A.....  
 Haplotype 2 .....F.....S.....V.....A.....E..

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## REFERENCES CITED IN TABLES A2.3, A4.1, A4.2

- Baudon D, Robert V, Roux J, Stanghellini A, Gazin P, Molez JF, Lhuillier M, Sartholi JL, Saluzzo JF, Cornet M & et al. (1984) Epidemic yellow fever in Upper Volta. *Lancet* 2: 42. doi:10.1016/s0140-6736(84)92027-0.
- Berger TO (1975) International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates U. S. Department of Health, Education, and Welfare, Public Health Service.
- Christophers R (1960) *Aedes Aegypti* (Linneaus), the Yellow Fever Mosquito. Cambridge University Press, New York.
- Chung HH, Cheng IC, Chen YC, Lin C, Tomita T & Teng HJ (2019) Voltage-gated sodium channel intron polymorphism and four mutations comprise six haplotypes in an *Aedes aegypti* population in Taiwan. *PLoS Negl Trop Dis* 13: e0007291. doi:10.1371/journal.pntd.0007291.
- Gillies MT & Coetzee M (1987) A supplement to the *Anophelinae* of South of the Sahara (Afrotropical region). Publications of the South African Institute for Medical Research.
- Hartberg WK & Gerberg EJ (1971) Laboratory colonization of *Aedes simpsoni* (Theobald) and *Eretmapodites quinquevittatus* Theobald. *Bull Wld Hlth Org* 43: 850-852.
- Hoogstraal H & Knight KL (1951) Observations on *Eretmapodites silvestris conchobius* Edwards (Culicidae) in the Anglo-Egyptian Sudan. *Am J Trop Med Hyg* s1-s33: 659 - 664.
- Huang Y-M (1997) The Subgenus *Stegomyia* of *Aedes* in the Afrotropical Region. II. The *Dendrophilus* group of species (Diptera: Culicidae). Smithsonian Institution, Department of Entomology, Washington DC.
- Linss JGB, Brito LP, Garcia GA, Araki AS, Bruno RV, Lima JBP, Valle D & Martins AJ (2014) Distribution and dissemination of the Val1016Ile and Phe1534Cys kdr mutations in *Aedes aegypti* Brazilian natural populations. *Parasit Vectors* 7: 25. doi:10.1186/1756-3305-7-25.

- Long KC, Ziegler SA, Thangamani S, Hausser NL, Kochel TJ, Higgs S & Tesh RB (2011) Experimental transmission of Mayaro virus by *Aedes aegypti*. *Am J Trop Med Hyg* 85: 750-757.
- Maxwell CA, Curtis CF, Haji H, Kisumku S, Thalib AI & Yahya SA (1990) Control of Bancroftian filariasis by integrating therapy with vector control using polystyrene beads in wet pit latrines. *Trans R Soc Trop Med Hyg* 84: 709-714. doi:10.1016/0035-9203(90)90158-b.
- McIntosh BM, Jupp PG, Anderson D & Dickinson D (1973) Attempts to transmit virus from seven species of mosquito. *J South Vet Ass* 44: 57-60.
- Mulwa F, Lutomiah J, Chepkorir E, Okello S, Eyase F, Tigoi C, Kahato M & Sang R (2018) Vector competence of *Aedes bromeliae* and *Aedes vitattus* mosquito populations from Kenya for chikungunya virus. *PLoS Negl Trop Dis* 12: e0006746. doi:10.1371/journal.pntd.0006746.
- Pedersen EM, Kilama WL, Swai AB, Kihamia CM, Rwiza H & Kisumku UM (1999) Bancroftian filariasis on Pemba Island, Zanzibar, Tanzania: an update on the status in urban and semi-urban communities. *Trop Med Int Health* 4: 295-301. doi:10.1046/j.1365-3156.1999.00391.x.
- Saavedra-Rodriguez K, Maloof FV, Campbell CL, Garcia-Rejon J, Lenhart A, Penilla P, Rodriguez A, Sandoval AA, Flores AE, Ponce G, Lozano S & Black WCt (2018) Parallel evolution of vgsc mutations at domains IS6, IIS6 and IIIS6 in pyrethroid resistant *Aedes aegypti* from Mexico. *Sci Rep* 8: 6747. doi:10.1038/s41598-018-25222-0.
- Saavedra-Rodriguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores AE, Fernandez-Salas I, Bisset J, Rodriguez M, McCall PJ, Donnelly MJ, Ranson H, Hemingway J & Black WCt (2007) A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. *Insect Mol Biol* 16: 785-798. doi:10.1111/j.1365-2583.2007.00774.x.

- Service MW (1990) Handbook to the Afrotropical Toxorhynchitine and Culicine mosquitoes, excepting *Aedes* and *Culex*. British Museum (Natural History), London, UK.
- Stenhouse SA, Plernsub S, Yanola J, Lumjuan N, Dantrakool A, Choochote W & Somboon P (2013) Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* (Diptera: Culicidae) by allele-specific PCR assay, and its distribution and effect on deltamethrin resistance in Thailand. *Parasit Vectors* 6: 253. doi:10.1186/1756-3305-6-253.
- Subra R (1981) Biology and Control of *Culex pipiens quinquefasciatus* Say, 1823 (Diptera:Culicidae) with Special Reference to Africa. *Insect Sci. Appl.* 1: 319-338.
- Turell MJ, Linthicum KJ, Patrican LA, Davies FG, Kairo A & Bailey CL (2008) Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. *J Med Entomol* 45: 102-108. doi:10.1603/0022-2585(2008)45[102:vcosam]2.0.co;2.
- Villanueva-Segura K, Ponce-Garcia G, Lopez-Monroy B, Mora-Jasso E, Perales L, Gonzalez-Santillan FJ, Ontiveros-Zapata K, Davila-Barboza JA & Flores AE (2020) Multiplex PCR for simultaneous genotyping of kdr mutations V410L, V1016I and F1534C in *Aedes aegypti* (L.). *Parasit Vectors* 13: 325. doi:10.1186/s13071-020-04193-0.
- Wilkerson RC, Linton Y-M, Fonseca DM, Schultz TR, Price DC & Strickman DA Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini that Balances Utility with Current Knowledge of Evolutionary Relationships. *Plos one* 10: e0133602.